GENETICS OF MAKAPUNO: A GENETIC TUMOR OF THE COCONUT ENDOSPERM

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ABSTRACT

The genetic misregulation resulting in the continued cell proliferation and uncontrolled growth of the mutant coconut endosperm (makapuno) was studied. Biochemical, cytological and histological data indicate that the makapuno condition resulted from the altered transcription of the genes for the enzymes involved in hormone metabolism. The altered hormone metabolism caused the misregulation of galactomannan metabolism which in turn effected the abnormal cell behavior and properties of the makapuno endosperm.

Introduction

The mutant coconut endosperm, locally known as makapuno, results from continued cell proliferation and uncontrolled growth, characteristic of neoplastic tissues or tumor. Early studies of Torres (1937) and Zuñiga (1953) indicate that the makapuno condition is gene-controlled, specifically one major gene effect. However, considering the several types of makapuno one can observe (Adriano and Manahan, 1931) and the series of biochemical processes the cells would have to undergo before continuous cell proliferation happens, it is quite clear that a major gene effect might be an oversimplification.

Genetic tumor is known to be inducible in plants. For example, certain interspecific hybrids in *Nicotiana (N. glauca* x *N. langsdorffii)* result in the formation of tumorous tissues quite identical to that of makapuno (Chuja, 1965; 1968). The same genetic tumors were induced by interspecific hybridization in *Datura* and by inbreeding in sweet clover (Braun, 1981). This malignant process is due to a single type of heritable cellular change that involves the regulatory mechanisms. Except for makapuno, there are no known naturally occurring genetic tumors in plants.

This study has the following objectives:

1. To elucidate the genetic systems responsible for the makapuno trait;
2. To induce the makapuno phenotype *in vitro* and *in vivo*.

Histological and Cytological Studies

Comparison of the normal and the makapuno tissues and cells show significant differences in cell organization, cell shape as well as cell sizes (Fig. 1).

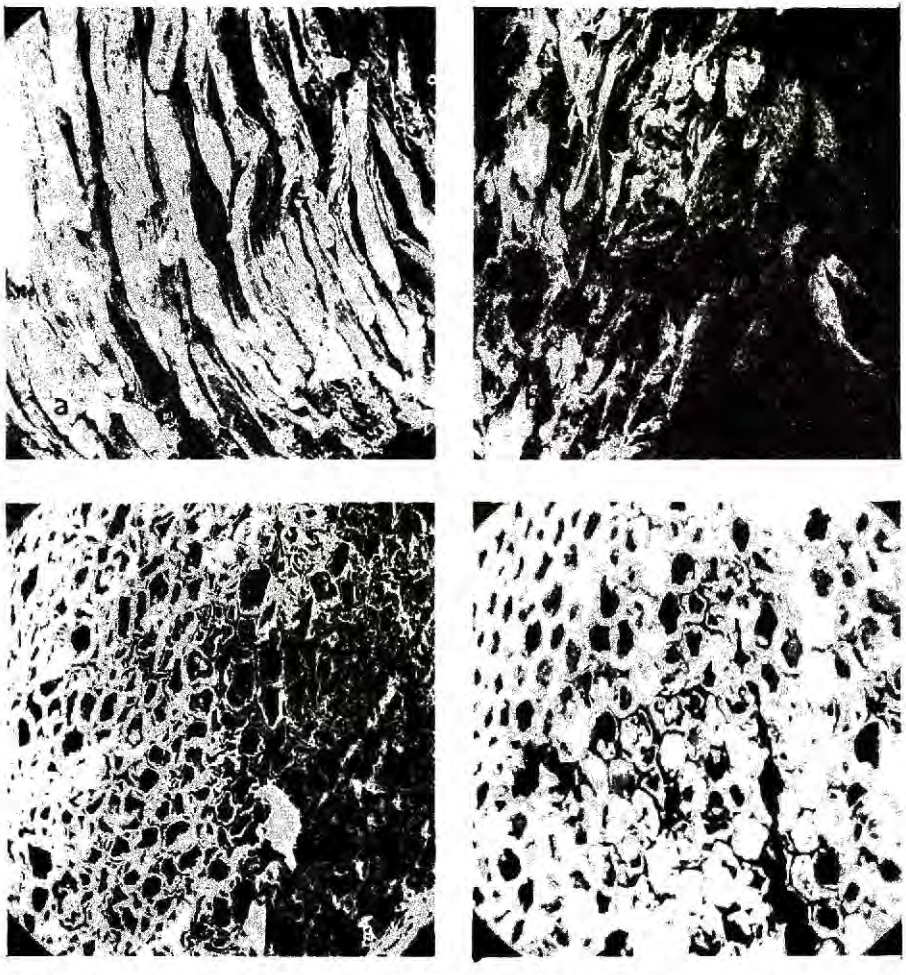

Fig. 1. Scanning electron micrographs of mature normal (a) and makapuno (b) endosperms, longitudinal section, 100x; Scanning electron micrographs of normal (c) and makapuno (d) endosperms, cross section, 150x.

Normal	Makapuno
1. Regular pattern of cell arrangement; cell adhesion	1. Irregular cell arrangement; no cell adhesion
2. Regular, longitudinal cell shape	2. Irregular
3. More or less the same cell sizes and are smaller	3. Varying cell sizes and are larger
4. Chromosome number predominantly 48 (3n) and fewer cells of higher ploidy	4. Chromosome number predominantly of higher ploidy
5. Normal cytokinesis	5. Cell division by budding

Bioassay for Tumor-inducing Substances

To find out whether the "abnormal" cell behavior of the makapuno can be induced in other plant tissues, White's medium to which extracts from both the normal and the makapuno endosperms were added, were used to culture carrot tissues.

The results show that the cell behavior of the makapuno was exhibited by the carrot tissues cultured in the makapuno plus medium. This means that there are some tumor-inducing substances in the makapuno which do not exist in the normal coconut endosperm.

The abnormality could be a consequence of the disturbance of the auxin-cytokinin balance.

Biochemical Studies

The biochemical studies of makapuno aim to elucidate the mechanisms that control the abnormal growth of cells in the mutant endosperm. The ultimate goal is to find out if there are altered gene products, and if there are, how they effect the mutant phenotype.

As suggested earlier in the bioassay studies, the abnormal endosperm could be the consequence of a disturbance in the auxin-cytokinin balance. It is possible, therefore, that a misregulation in IAA (indole-β-acetic acid) metabolism is one of the key factors in producing the makapuno endosperm. The three enzymes studied are: peroxidase, catalase and trytophan-α-ketoglutarate aminotransferase.

Peroxidase is involved in the degradation of IAA because of its IAA oxidase

Peroxidase is involved in the degradation of IAA because of its IAA oxidase activity, whereas tryptophan α-ketoglutarate aminotransferase is involved in IAA biosynthesis. Catalase is associated with peroxidase inhibition.

Peroxidase

The peroxidase from mature makapuno and normal coconut endosperms were isolated, purified and characterized according to molecular weight, charge, mobility, temperature and pH dependence, saturation kinetics and temperature stability. In general, the peroxidases from normal and makapuno endosperms exhibit similar physicochemical properties.

Peroxidase specific activities and isoenzyme pattern at different stages of endosperm development were investigated. Significant differences in the specific activities were noted (Table 1). Makapuno peroxidases exhibited significantly high activity at maturity when compared to the normal.

It was also observed that no isoenzyme was unique to either endosperm type. The number of isoenzymes for both was the same at any given age but it decreased with age.

Table 1. Peroxidase activity of coconut endosperms at various stages of development

Stage	*Age (mo)*	*Specific activity (units/mg protein)**		
		Normal	*Makapuno*	*Genotype x Age*
II	7-8	0.098^{a}	0.50^{ab}	s
III	8-9	0.27^{b}	0.26^{bc}	ns
IV	9-10	0.12^{b}	0.79^{a}	s
V	10-11	0.18^{b}	0.08^{c}	ns
VI	11-12	0.67^{b}	0.13^{c}	s

*Average of 3 replications.
From Mujor and Ramirez, 1980.

L-tryptophan-α-ketoglutarate aminostransferase

Both the normal and the makapuno endosperm exhibit L-tryptophan-α-ketoglutarate aminotransferase activities. This enzyme catalyzes IAA biosynthesis from tryptophan via the indole-3-pyruvic acid pathway. Ontogenetic studies in the normal endosperm showed that aminotransferase activity declined from the first stage of endosperm formation until maturity (Fig. 2). In contrast, the activity in the makapuno was higher at the first stage, slightly declined at the second stage, increased at the third, significantly reduced to 0 at the fourth stage and abruptly increased until the sixth stage of endosperm formation. It was noted that at the onset of tumorigenesis (9-10 month stage) in makapuno is marked by a sudden decrease in IAA level. Therefore, it appears that the makapuno condition is a product of an aberrant gene regulation involving IAA synthesis and/or degradation.

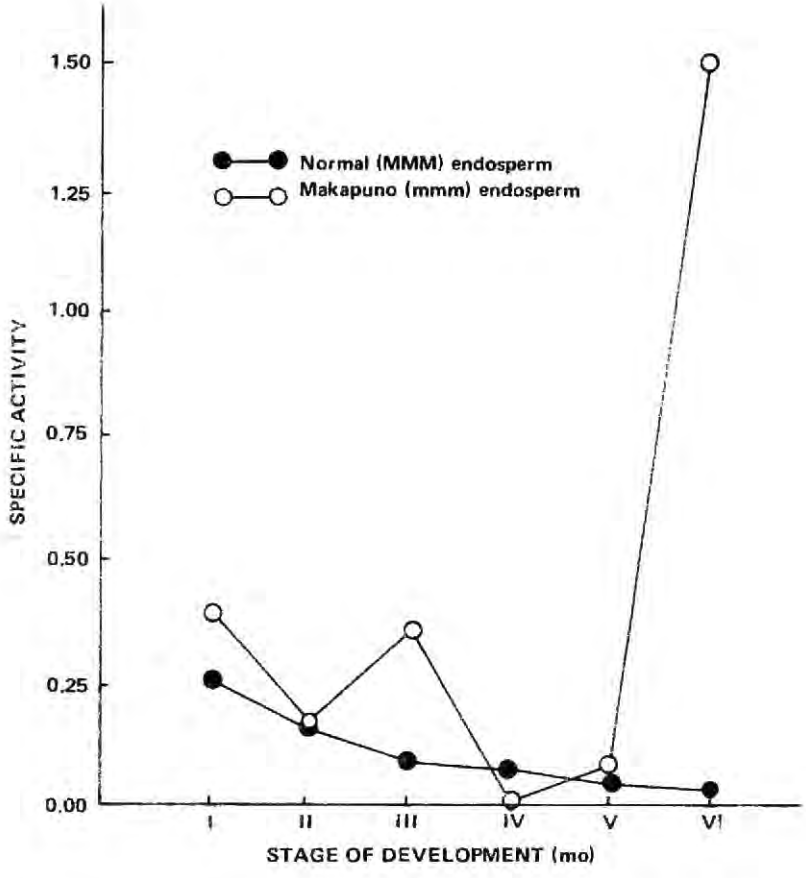

Fig. 2. Ontogenetic activity of tryptophan aminotransferase in the normal and makapuno coconut endosperms.
From Lardizabal, 1980.

Catalase

Catalases inhibit peroxidase activity. Catalase activity is reported to be greatly lowered in tumorous tissues. To investigate the role of catalase in abnormal growth of makapuno endosperm and its relationship with peroxidases, this enzyme was also characterized. Results showed that catalase activity was significantly higher in makapuno than in the normal endosperms at all stages of growth (Table 2). Five anodic isoenzymes were detected in the makapuno endosperm. On the other hand, the normal endosperms showed four isoenzymes, except at stage 1 which had only three.

Table 2. Specific activity of catalase from normal and makapuno coconut endosperms at different stages of development (Padre, R.A., 1980)

Stage	*Age (mo)*	*Specific activity (units/mg protein)*	
		Normal	*Makapuno*
I	6-7	3.15	11.90
II	7-8	2.15	4.05
III	8-9	2.40	3.05
IV	9-10	1.40	3.00
V	10-11	2.35	8.20
VI	11-12	2.21	4.90

From Padre, 1980

Adenosine cyclic monophosphate (Cyclic AMP)

The levels of cAMP in the normal and makapuno endosperms were compared ontogenetically. Makapuno exhibited significantly lower levels at all stages when compared with the normal endosperms (Table 3). Among the normal endosperms, the level of cAMP was constantly high at stages I to V and considerably low at stage VI; no marked differences were noted at stages I to IV. It is postulated that the low level of cAMP in makapuno is related to the level of endogenous auxins in this endosperm.

Table 3. Cyclic AMP levels* in the normal and mutant (makapuno) coconut endosperms during development

Stage	*Age (mo)*	*Endosperm genotypes*		
		Makapuno (mmm)	*Normal (M– –)*	*Normal (MMM)*
I	6-7	0.89	4.4	4.6
II	7-8	0.69	5.4	4.8
III	8-9	0.40	4.6	4.7
IV	9-10	0.91	5.9	4.8
V	10-11	0.04	4.1	3.4
VI	11-12	0.03	1.3	1.3

*Average of 2 replications (PMQL/mg fresh wt)
From Tanchuco *et al.*, 1981

Proximate Analyses

Comparative analyses of chemical constituents of makapuno and normal coconut endosperms at different stages of growth were also undertaken (Tables 4, 5, 6, 7 and 8).

Table 4. Comparative chemical analyses of makapuno and normal coconut endosperm at various stages of development[a]

Sample	*% Starch*					
	Age (months)					
	6	*7*	*8*	*9*	*10*	*11*
Makapuno	3.60	3.60	6.13	9.14	11.23	11.13
Normal		5.74	8.52	8.36	9.91	10.31
Tree			ns	*	ns	ns
Trt x Tree				ns	ns	ns
CV(%)			19.4	18.2	10.8	14.3

[a]Absence of statistical analysis for 6 and 7 month-old samples indicate lack of data.

Table 5. Comparative chemical analyses of makapuno and normal coconut endosperm at various stages of development[a]

Sample	*% Sugars*					
	Age (months)					
	6	*7*	*8*	*9*	*10*	*11*
Makapuno	60.0	47.9	19.7	10.9	5.9	5.5
Normal		25.0	17.4	12.4	9.1	6.6
Tree			ns	**	ns	ns
Trt x Tree			*	**	*	ns
CV (%)			15.7	14.3	9.2	21.2

[a]Absence of statistical analysis for 6 and 7 month-old samples indicate lack of data.

Table 6. Comparative chemical analyses of makapuno and normal coconut endosperm at various stages of development[a]

Sample	*% Fat*					
	Age (months)					
	6	*7*	*8*	*9*	*10*	*11*
Makapuno	4.25	8.62	34.72	40.31	42.8	47.0
Normal	8.30	27.23	27.58	36.02	39.1	43.5
Tree		**	ns	ns	ns	ns
Trt x Tree		**	ns	*	ns	ns
CV (%)		5.0	31.7	14.0	14.3	5.4

[a]Absence of statistical analysis for 6 and 7 month-old samples indicate lack of data.

Table 7. Comparative chemical analyses of makapuno and normal coconut endosperm at various stages of development

Sample	*% Proteins*					
	Age (months)					
	6	*7*	*8*	*9*	*10*	*11*
Makapuno	11.9	9.70	7.95	7.92	7.92	7.33
Normal		8.96	8.06	8.15	8.07	7.57
Tree			ns	ns	ns	ns
Trt x tree			ns	**	ns	ns
CV (%)			10.4	9.9	8.3	7.6

[a]Absence of statistical analysis for 6 and 7 month-old samples indicate lack of data

Table 8. Amino acid composition of dried defatted coconut and makapuno endosperms (g amino acid/100 g protein)[a]

Amino acid	*Normal coconut*		*Makapuno*		
	9 mo.	*11 mo.*	*7 mo.*	*9 mo.*	*11 mo.*
Lysine	4.48 a	4.00 b	4.58a	4.35 ab	4.02 b
Histidine	1.86	2.17	1.52	1.77	1.88
Ammonia	1.23	1.51	2.09	1.71	1.34
Arginine	11.84 b	11.48bc	8.42 d	11.33 c	13.76a
Aspartic acid	8.13	8.43	8.49	7.69	8.98
Threonine	3.76	3.55 a	2.59 b	2.99 b	3.72 a
Serine	4.59 bc	4.94 ab	4.44 c	4.70 abc	5.02 a
Glutamic acid	20.46 a	20.73 a	16.93 b	21.15 a	9.99 c
Proline	3.32	2.78	3.01	3.47	3.52
Glycine	4.25 ab	4.36 a	3.62 c	3.86 bc	4.45 a
Alanine	5.36 b	4.78 c	9.18 a	5.49 b	5.21 b
Cysteine	1.86 ab	1.67 ab	1.51 b	1.88 ab	1.92 a
Valine	5.64 ab	5.91 a	4.70 c	5.18 bc	5.32 b
Methionine	1.56 b	1.64 ab	1.49 b	1.53 b	1.94 a
Isoleucine	3.63 ab	3.80 a	3.27 b	3.31 ab	3.72 ab
Leucine	6.26 a	6.51 a	5.20 b	5.45 b	6.51 a
Tryptophan	0.85 ab	0.91 a	0.83ab	0.63 b	0.70 ab
Phenylalanine	3.91 ab	4.07 a	2.87 c	3.57 b	4.01 ab
Tyrosine	ND	ND	ND	ND	ND
Chemical Score, %	81	73	65	63	70
Limiting Amino Acid	Lysine	Lysine	Threonine	Tryptophan	Tryptophan

[a]Means in a horizontal row followed by common letters are not significantly different at 0.05 level of significance (DMRT).

In general, there were no significant differences between values for starch, free sugars, fat and protein obtained for normal and makapuno endosperms of different ages. However, ontogenetic changes were observed for some components. Proteins and starch did not change significantly from 7 to 11 months of the normal endosperm. On the other hand, the makapuno endosperm showed a decrease in protein level as the nut matured (from 11.9% to 7.3%) while starch increased (from 3.6% to 11.13%). Free sugars decreased in both samples but a more dramatic change was observed in makapuno (90% vs. 75%). Fat content increased in both samples, but again more dramatically in makapuno.

In amino acid composition, the mature makapuno had higher arginine, higher alanine, 50% less glutamic acid and lower valine values than normal coconut endosperms, indicating that makapuno proteins are generally more basic than those of the normal coconut.

Carbohydrates

Although proximate analyses of normal and makapuno endosperms did not show significant differences, fractionation of crude extracts with $(NH_4)_2SO_4$ resulted in separation of viscous substances which are present 36 times greater in makapuno than in the normal (Table 9). In makapuno, the viscous components increased with age, reaching their peak at endosperm maturity. In contrast, the viscous components decreased with age in the normal endosperm. Proximate analyses showed a 14.4% polysaccharides content for the normal endosperm and 7.5% for the makapuno viscous components.

By paper chromatography, the monosaccharides galactose and mannose were detected at all stages of development in both endosperm types, indicating the

Table 9. Amount of viscous precipitate (G) among normal and makapuno coconut endosperms at various stages of development

Stage	*Age (mo)*	*Genotype**		
		MMM	*M – –*	*mmm*
II	7-8	0.85 a	1.18 a	0.42 a
III	8-9	1.14 a	0.94 ab	1.74 b
IV	9-10	0.89 a	0.88 ab	1.52 b
V	10-11	0.10 b	0.62 bc	2.23 c
VI	11-12	0.08 b	0.26 c	2.68 d

*Average of 3 replications.
Means followed by the same letter are not significantly different from each other at 0.05 level of significance (DMRT).
From Mujer *et al.*, 1983b.

continued synthesis of galactomannans from the onset of endosperm formation until maturity. The viscous cellular components may have been a consequence of a misregulated genetic system that occurred earlier in the course of development. The end product(s) of this system probably alter still some pathways leading to the biosynthesis/degradation of these cellular components whose level is critical to the expression of the normal phenotype. One possible pathway is that of galactomannan degradation. The presence of this disaccharide in very high amounts in makapuno suggests that the pathway leading to its degradation is shut off. This is in contrast to the normal endosperm whose galactomannan degradation continues. Galactomannan is an important cell wall constituent in plants. Its altered level could explain the absence of cell adhesion in makapuno.

Galactomannan degradation

The accumulation of the viscous component in the makapuno could be due to the inactivation or absence of some enzymes that normally catalyze its degradation to non-viscous products. When crude extracts from the normal endosperm were added to the makapuno viscous components, an 82% reduction in relative viscosity was observed (Table 10).

Table 10. Relative viscosity of viscous component (10% solution from makapuno before and after addition of crude extract from the normal endosperm

	Treatment	*Incubation time (hr.)*	*Relative viscosity*	*% Decrease in relative viscosity*
1.	0.50% $(NH_4)_2SO_4$ fraction	0	2.39	
	+ crude extract	20	0.43	82.0
2.	0.50% $(NH_4)_2SO_4$ fraction	0	2.24	
	+ H_2O	20	2.03	9.6

From Mujer *et al.*, 1983b

Galactomannan degradation requires at least three different enzymes: α-D-galactosidase for the removal of the (1 → 6) α-D-galactose side chains; β-D-mannanase for fission of the (1 → 4)-β-D-mannan backbone into oligosaccharides; and β-D-mannosidase for complete hydrolysis of the D-manno-oligosaacharides to D-mannose.

1. α-D-galactosidase

Table 11 shows the α-D-galactosidase activity at various stages of normal endosperm development. The enzyme activity continually increased with age until endosperm maturity. The pattern of α-D-galactosidase activity is inversely correlated with the amount of galactomannans in the developing normal endos-

perm. Hence, it is possible that this enzyme plays a major role in the galactomannan degradation.

Table 11. α-D-galactosidase activity in normal coconut endosperm at various stages of development

Age (Mo. after pollination)	*Specific activity* (milliunits/mg protein)*
7-8	0.07 d
8-9	0.23 d
9-10	0.59 c
10-11	0.99 b
11-12	1.71 a

*Average of 6 replications. Means followed by the same letter are not significantly different at 0.05 level (DMRT).
From Mujer *et al.*, 1984b

In sharp contrast, α-D-galactosidase activity was not detected in the makapuno endosperm when the usual kinetic assay procedure was employed. However, activity was detected when the enzyme was incubated in the reaction mixture for 18 to 24 hours at 30°C (Incubation period for the normal, 30 min.) α-D-galactosidase activity was 8,268-fold lower in makapuno as compared to that of the normal endosperm.

There are three possible causes of these differences in α-D-galactosidase activities of the normal and makapuno endosperm:

1. there is a continuous repression of enzyme synthesis;
2. the presence or absence of specific effectors caused the deficiency in enzyme activity;
3. the α-D-galactosidase in the makapuno is enzymatically/catalytically defective.

To determine the specific cause, the enzyme was purified and characterized. The results show that the α-D-galactosidase from normal and makapuno endosperm are identical. Hence, the deficiency of activity in makapuno is not due to a structural mutation of the α-D-galactosidae gene, but probably to either a continuous repression of enzyme synthesis or the presence of specifice effectors.

2. β-D-mannanase

The β-D-mannanase activity was found to decrease in maturing endosperm for both the normal and makapuno (Table 12). It was higher in the makapuno than the normal at several stages. These results indicate an active degradation going on in both types of endosperms during their maturity. The higher mannanase activity in makapuno could account for its shorter and more viscous galactoman-

Table 12. β-D-Mannanase activity in normal and makapuno endosperm at various stages of development (milliunits/mg protein)

Age (mo. after pollination)	*Genotype* *MMM (normal)*	*mmm (makapuno)*
6-7	69.38 d	–
7-8	28.31 e	60.02 a
8-9	29.14 e	48.01 a
9-10	17.21 e	36.08 b
10-11	16.93 e	41.63 a
11-12	16.10 e	2.22 c

Average of 3 replicates. Values followed by the same letter are not significantly different at 0.05 level (DMRT).
From Dela Cruz, N. 1985

nans. The increased levels of galactomannans in the maturing makapuno endosperm could induce the higher activity of this enzyme in makapuno.

β-D-mannanase from normal and makapuno endosperms also exhibited identical properties.

3. β-D-mannosidase

Table 13 shows that the specific activities of β-D-mannosidase decreased in maturing normal solid endosperms, while they increased in the liquid endosperm

Table 13. β-D-mannosidase activity of normal and makapuno endosperm during maturation (milliunits/mg protein)

Age (mo. after pollination)	*Genotype* *MMM (Normal)*	*mmm (makapuno)*
A. Solid endosperm		
7-8	1.39	0.19
8-9	0.59	0.10
9-10	0.44	n.c.
10-11	0.26	n.c.
11-12	0.28	n.c.
B. Liquid endosperm		
7-8	0.16	0.13
8-9	0.14	0.11
9-10	0.18	0.12
10-11	0.68	0.21
11-12	1.49	0.21

*n.c., no color formed but high absorbance values due to turbidity.
From Mendoza *et al.*, 1985

of the maturing nuts. The decrease in activity of β-D-mannosidase in both normal and makapuno endosperms indicates less amounts of energy sources and cell metabolites. It further indicates the slowing down of metabolism in the maturing cells.

Like the α-D-galactosidase and β-D-mannanase, the chemical properties of β-D-mannosidase in the normal and makapuno endosperms are identical.

The results point to a bigger role of the α-D-galactosidase gene in the expression of the makapuno phenotype. A summary of the series of events that lead to the makapuno endosperm is shown in Fig. 3.

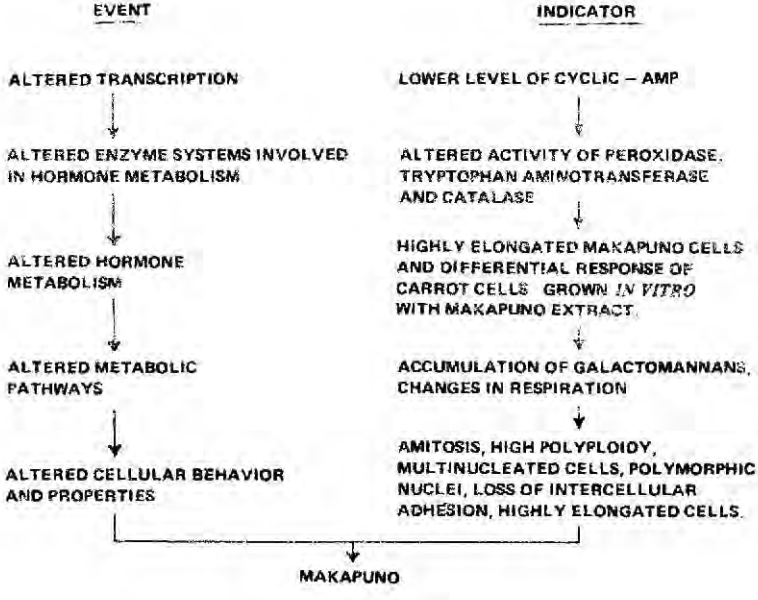

Fig. 3. Genetic misregulation in makapuno.

The detection of α-D-galactosidase activity in makapuno rules out the possibility of a forbidden mutation in or absence of the structural gene for α-D-galactosidase in this endosperm. The fact that the enzyme exhibited catalytic properties similar to those from the normal suggests that either a continuous repression of enzyme synthesis or the presence/absence of specific effectors caused the deficiency in enzyme activity.

The deficiency of α-D-galactosidase activity coincides with the significantly high level of galactomannans in makapuno. Possibly the normal degradation pathway is disturbed in this tissue, consequently leading to the accumulation of high amounts of galactomannans. In turn, the abnormally high galactomannan level have caused the expression of altered characteristics, such as loss of intercellular adhesion, highly elongated cells and amitosis. Balasubramaniam (1976) pointed to the structural role of galactomannans in the formation of the primary cell wall of coconut endosperms. They act as the matrix polysaccharide and together with glusans form the initial cell wall layers. Rao and Mukherjie (1962) reported that galactomannans were dominant in the cell walls of unripe palmyra palm seeds, whereas those of the ripe seeds consisted chiefly of mannans containing a small percentage of galactose residues. This means that most galactose groups are removed during seed maturation. The process occurs with the transition of the endosperm from the hydrated gelatinous phase to the dehydrated solid mature state. The almost virtual absence of galactose hydrolysis due to α-D-galactosidase deficiency could have blocked the normal endosperm development in the makapuno.

Inhibition of α-D-galactosidase activity

The inhibitory effects of various sugars and related compounds on α-D-galactosidase activity were tested. The inhibition follows the order: D-galactose > myo-inositol > D-glucose-6-phosphate > L-arabinose > melibiose (Tables 14 and 15).

The ability of galactose to strongly inhibit α-D-galactosidase may yet find its application in the artificial induction of makapuno. Exogenous incorporation of this inhibitor or its analogues to the growth medium of normal endosperms culture *in vitro* or its direct application to normal coconut may lead to the formation of the makapuno endosperms.

Artificial Induction of Makapuno

Two approaches were used in the artificial induction of makapuno conditions. (1) *in vitro* induction on cultured tissues of the normal endosperm, and (2) *in vivo* induction on developing nuts.

In vitro induction

The *in vitro* induction requires successful tissue culture of the normal coconut endosperm. Callus was successfully induced in explants from young coconut endosperms (Fig. 4). The callus cells are similar to the normal endosperm cells in shape, thickness of cell wall and general appearance, although larger than the normal and makapuno cells. The callus cells exhibited amitotic division in the form of budding nuclei similar to those observed in makapuno cells, although the normal cytokinesis predominated. In addition, multi-nucleated cells and cells of different

Table 14. Inhibition of α-D-galactosidase A-catalyzed hydrolysis of p-nitrophenyl α-D-galactoside by sugars and related compounds at 30°C

Inhibitor	*Conc. of inhibitor (M)*	*Inhibition (%)*
D-Arabinose	75	3
L-Arabinose	75	35
	50	25
	25	20
	5	8
L-Fucose	75	9
D-Galactose	5	88
	1	68
	0.5	46
	0.1	24
D-Glucose	75	5
D-Glucose-6-phosphate	75	60
	50	42
	25	22
	5	4
D-Mannose	75	3
	25	0
Myo-inositol	75	86
	50	80
	25	67
	5	26
Melibiose	75	25
Sucrose	75	0
Coconut endosperm galactomannans	0.005%	5
	0.010%	7
	0.025%	24

From Mujer *et al.*, 1984b

Table 15. Inhibition of α-D-galactosidase A by cations and sulfhydryl-specific reagents at 30°C

Inhibitor	*Concn. of inhibitor (M)*	*Inhibition (%)*
Iodoacetic acid	0.075	96
	0.050	66
	0.025	27
	0.005	6
Iodoacetamide	0.075	0
Dithiothreitol	0.075	0
Mn^{2+}	0.002	0
K^+	0.5	0
Na^+	0.5	0

From Mujer *et al.*, 1984b

Fig. 4. Four-month old callus induced from normal coconut endosperm.

ploidy level were observed in the calli. Nuclei counts ranged from 1 to 5 nuclei per cell, while chromosome counts ranged from 2n(32) to 7n(112).

Our next move is to induce the makapuno condition in the calli. In the biochemical studies, it was shown that the makapuno has very negligible α-D-galactosidase activity while the normal exhibits high activity. Furthermore, α-D-galactosidase activity can be inhibited by feed back inhibition; D-galactose, myo-inositol, D-glu-6-P, L-arabinose and melibiose are among the inhibitors. These inhibitors are being added to culture medium of the endosperm calli.

In vivo induction

This study focused on treatment of young coconuts with the inhibitors introduced into the endosperms by: (a) injection in the peduncles at regular intervals and (b) continuous application by gravity through the peduncle. Distilled water was used as control.

The D-galactose treated nuts exhibited irregular endosperm surface while the control nuts had smooth endosperm surfaces.

Literature Cited

Adriano, F.T. and M. Manahan. 1931. The nutritive value of green, ripe and sport coconut (buko, niyog and makapuno). *Philipp. Agric.* **20**:195-198.

Agaton, O.C., D.A. Ramirez and E.M.T. Mendoza. 1985. Cytology of callus induced *in vitro* from normal coconut endosperm. *Kalikasan: Philipp. J. Biol.* in press.

Ahuja, M.R. 1965. Genetic control of tumor formation in higher plants. *Q. Rev. Biol.* **40**:329-340.

_____. 1968. A hypothesis and evidence concerning the genetic components controlling tumor formation in *Nicotiana. Mol. Genetics.* **103**:176-184.

Balasubramaniam, K. 1976. Polysaccharides of the kernel of maturing and matured coconuts. *J. Food Science* **41**:1370-1373.

Braun, A.C. 1978. Plant Tumors. *Biochem. Biophys. Acta.* **516**:167-191.

Deangkinay, G.P. and D.A. Ramirez. 1971. Action of the makapuno gene (*m*) in coconut. I. Effect of coconut milk supplement on cellular behavior of carrot tissue cultures. *Philipp. Agric.* **54**:391-396.

Dela Cruz, N. 1985. The ontogeny of β-D-mannanase in the mutant (makapuno) and normal coconut endosperms. B.S. thesis. University of the Philippines at Los Baños.

Dela Cruz, S.S. and D.A. Ramirez. 1968. The cytology of the developing endosperms of the normal and the mutant (makapuno) coconut. *Philipp. Agric.* **52**:72-81.

Hautea, R.A. 1983. Electrophoretic patterns of soluble endosperm proteins from 11 to 12 month-old makapuno and normal coconuts. B. S. thesis. University of the Philippines Los Baños. 47 p.

Institute of Plant Breeding Annual Reports. 1979, 1980, 1981, 1982, 1983, 1984, 1985.

Lardizabal, F.C. 1980. Tryptophan aminotransferase activity in the normal and makapuno coconut endosperm development. B.S. thesis. University of the Philippines at Los Baños. 37 p.

Mamaril, A.C. 1982. Electrophoretic patterns of soluble endosperm proteins from 8 to 9 month-old makapuno and normal coconuts. B.S. thesis. University of the Philippines, Los Baños, 63 p.

Mendoza, E.M.T., J. L. Samonte, C.V. Mujer, N.B. dela Cruz and D.A. Ramirez. 1985. Galactomannan metabolism in developing normal and makapuno coconut endosperm. *Trans. Nat. Acad., Sci. & Tech.* (Phils) 7:237-247.

Mujer, C.V. and D.A. Ramirez. 1980. Peroxidase isoenzymes in the mutant (makapuno) and normal endosperms of coconut. *Kalikasan Philipp. J. Biol.* **9**:25-30.

_____, E.M.T. Mendoza and D.A. Ramirez. 1983a. Coconut peroxidase isoenzymes; isolation, partial purification and physicochemical properties. *Phytochemistry* **22**: 1335-1340.

_____, A.S. Arambulo, E.M.T. Mendoza, and D.A. Ramirez. 1983b. The viscous components of the mutant (makapuno) coconut endosperm. I. Isolation and characterization.

_____, D.A. Ramirez and E.M.T. Mendoza. 1984a. α-D-galactosidase deficiency in coconut endosperm; its possible pleiotropic effects in makapuno. *Phytochemistry* **23**: 893-894.

_____. 1984b. Coconut α-D-galactosidase isoenzymes: isolation, purification and characterization. *Phytochemistry* **23**:1251-1254.

Oliveros, A.M.R. 1983. Electrophoretic pattern of soluble endosperm proteins from 9 to 10 month-old makapuno and normal coconuts. B.S. Thesis. University of the Philippines Los Baños. 56 p.

Padre, R.A. 1980. Ontogeny of specific activity and isoenzymes pattern of catalase in normal and makapuno endosperm of coconut. B.S. Thesis. University of the Philippines Los Baños. 49 p.

Posa, B.T., D.A. Ramirez, and R.G. Bugayong. 1971. Action of the macapuno gene (*m*) in coconut. II. Protein-bound amino acids. *Philipp. Agric.* **54**:397-401.

Rao, C.V.N. and A.K. Mukherjee. 1962. Constitution of a mannan from the kernel of palmyra palm nut. *J. Indian Chem. Soc.* **39**:711-716.

Romero, G.O. 1983. Electrophoretic patterns of soluble endosperm proteins from 10 to 11 month-old normal and makapuno coconuts. B.S. Thesis. University of the Philippines, Los Baños. 52 p.

Tanchuco, J.Q., D.A. Ramirez, and C.V. Mujer. 1981. Comparative levels of cyclic AMP in the mutant (makapuno) and normal coconut endosperms. *Kalikasan. Philipp. J. Biol.* **10**: 351-354.

Torres, J. 1937. Some notes on macapuno coconut and its inheritance. *Philipp. Agric. J.* **8**: 27-37.

Zuñiga, L.C. 1953. The probable inheritance of the macapuno character of coconut. *Philipp. Agric.* **36**:402-413.