Effect of Fruit Age on Endosperm Type and Embryo Germination of Makapuno Coconut

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ABSTRACT

Makapuno, a mutant coconut of soft endosperm, often found in Laguna Tall of the Philippines, were classified as Type A = Few semisolid endosperm or leathery kernel obtained, Type B = When the semisolid endosperm fills 50% the cavity, Type C = When the semisolid endosperm almost fills the cavity. Almost all nuts of early harvest (around 9 month old) were of the A type and those harvested at the age of 10 months were of the B type. Late harvest (Eleven month old nuts) gave majority of the C type nuts. This indicated a relationship between nut age and expression of A, B and C type. Morphologically embryos of all three types were found identical. Weight, length and breadth of embryos showed an increasing trend with the increase of nut age. Percent germinations of embryos of 9, 10 and 11 months old nuts were almost similar. *In vitro* growth rate of embryos as well as growth of plantlets of 11 months old embryos was found to be superior compared to 9 and 10 months old embryos. However, the 9-month-old embryos are slow to bud break, but after germination, the rate of growth of plantlet is similar to the other types. More nuts can be harvested if physiological age of nut can be decreased at which embryo of A type endosperm nuts can be successfully rescued. The 9 month old embryos require a longer culture time.

Keywords: Coconut, Makapuno, embryo, *in vitro* germination

INTRODUCTION

Makapuno, a mutant coconut of soft endosperm, is often found in Laguna Tall of the Philippines. The endosperms of Makapuno are not always similar in character and different types have been reported. Adriano and Manahan (1931) described three different types of Makapuno endosperm such as A, B and C types based on how much liquid and soft meat fill the nut. Type A is slightly thicker and softer than that of normal coconut; the rest of the cavity is filled with thin liquid endosperm. In Type B, soft solid endosperm fills about 50% of the cavity; the rest is filled with thick liquid. In Type C, soft solid endosperm almost fills the cavity; about a spoonful of thick liquid may be present. Whether a particular type is borne by a tree during its productive life is still to be ascertained. Sometimes, different types are born by one tree while it has been established that Makapuno character is controlled by a single recessive gene (m) (Zuniga, 1953). The occurrence of at least three different types of Makapuno endosperm suggests that other factors may be influencing the expression of this character.

It is common knowledge that the embryo of a Makapuno nut does not germinate in situ due to rotting of the endosperm soon after nut reaches maturity (approximately 11 months). De Guzman and del Rosario (1964) showed that the Makapuno embryo is normal and viable and in fact can be rescued through in vitro culture. This Makapuno embryo culture technique developed by de Guzman has been improved and now being applied in many coconut laboratories worldwide. Over the years, the laboratories have reported variable percentages of embryo germination and seedling recovery. The question of whether these variable responses of the Makapuno embryo to *in vitro* culture result from genetic differences has been continuously asked. Hence, the study was undertaken to determine the effect of fruit age on expression of the types of Makapuno endosperm as well as on *in vitro* embryo germination.

MATERIALS AND METHODS

The fruits and embryos used in this experiment were collected from the Makapuno plantation at UPLB, Laguna, Philippines. All the plants were grown from in vitro cultured Makapuno embryos and subsequently established in the field during 70s'. Each individual palm is considered a unique genotype in our study, since the parents of the population were unknown.

Dates of opening of each inflorescences of every palm were tagged starting in March to June 2004. Fruits were harvested in February to May 2005, when they reached at the ages 9, 10 and 11months. Necessary data of harvested fruits were collected before dehusking. Nuts were splited and embryos were excised, noting down the age of nuts and types of endosperm. Length, breadth and weight of embryos were recorded, after which the embryos were prepared for the in vitro germination. Nuts from the regular harvest (once in every five weeks) of the untagged inflorescence were also used which were grouped into A, B and C endosperm types depending on how much of the endosperm filled the nuts (Adriano and Manahan, 1931) as follows.

Type A = Few semisolid endosperm or leathery kernel obtained

Type B = When the semisolid endosperm fills 50% the cavity

Type C = When the semisolid endosperm almost fills the cavity

Sterilized embryos were inoculated in growth medium for germination following the protocol used by the UPLB Makapuno Laboratory (Engelmann et al. 2002). Protrusion of the first scale leaf was considered as germination. Embryos that did not show any change in size were considered ungerminated.

F-test was used to test the homogeneity for means of treatments. A one sample χ 2 test was used to measure the amount of differences between the observed frequencies and the expected frequencies of a specific character.

RESULTS AND DISCUSSION

Fruit weight and size

Whole fruit weight (whole nut with husk), nut (without husk) and husks weight were not influenced by the age harvested at 9, 10 or 11 months. No significant differences were noticed in polar diameter, equatorial diameter, polar and equatorial circumferences of whole fruit when harvested at the age of 9, 10 and 11 months (Table 1). Harvesting ages did not affect weight as well as size of de-husking fruit (nut) (Table 2). The results were reflected in fruit components analysis (Fig 1). In normal coconut, volume and weight of nut reached maximum at the age of eight months. Subsequently, the fruit began to loose weight gradually due to reduction of water in the cavity through evaporation (Copeland, 1931). In Makapuno change of kernel to viscous substance restricts water loss through evaporation. Weight loss of fruit through maturation drying at age of 9, 10 and 11 months, respectively, might have insignificant. However, maturation drying in coconut usually starts at 11 months of age (Ohler, 1999). Thus, the fruit weight harvested at various ages might be observed similar (Menon and Pandalai, 1958 Child, 1974 and Veloso, 1983). Weight and size of nuts are varietal characters and attained maximum after certain ages. The population under study was derived from Laguna Tall of the Philippines whose parents were of diverged origin. Homogeneity in many characters was noticed in the population although critical analysis revealed substantial variations.

Type of Makapuno

Nut harvested at 9 months were 100 % of the A type and none of the B or C type. Those harvested at 10 months were of the B type. Eleven months old nuts showed 13 % B and 87 % C type and none of “A” type. While harvesting nuts at the age of 10 and 11 months it was observed that there was no “A” type Makapuno amongst the nuts (Fig 2). It appears from the result that the types of Makapuno are the effects of nut age. Although insignificant, some individual of the population did not produce C type when harvested at the age of 11months. A, B and C type endosperm developed progressively with the increase of nut age. It has been shown that in Makapuno, deficiency of α-D galactosidase gene allows synthesis and accumulation of water soluble galactomannans, which is responsible for the development of Makapuno endosperm (Mujer et al., 1984). Towards maturity the genotypes might influence the hormonal activity, which in turn affects the gene product and thus causes different types of Makapuno. In normal nut, enzyme products of α-D-galactosidase gene metabolize galactomannans to free galactose and insoluble mannan.

Intrinsic viscosity of galactomannans in Makapuno began to increase from 8 to 11 months of nut ages (Mendoza et al. 1985). This might be an evidence of progressive development of Makapuno from A, B and C types where B and C were physiologically much more different than A type.

Del Rosario and de Guzman (1981) reported a high level of cytokinin in Makapuno liquid endosperm which induces cell division and cell enlargement, as a result, increases the thickness of the endosperm and fills the nut cavity. Metabolism of the endosperm cell wall by cytokinin develops the Makapuno kernel they added. Since, hormonal activities become distinct with the maturity of living organisms, in Makapuno might have the same incidence of development from A to B to C types with the increase of nut age. Abraham et al. (1965) also reported a continued cell proliferation and uncontrolled cell growth of neoplastic or tumorous tissues in Makapuno coconut, which gradually fill the nut cavity.

Embryo size and weight

Weight and breadth of embryos in the three age groups found similar while the length was significantly longer in 11-month old nuts (Table 3). Higher weights of embryos were recorded in the Type C nuts followed by the Type B and the lowest weight found in Type A. Although, the lengths of embryos found to be statistically uniform in nuts of different types of endosperm, an increasing trend was noticed in A to B to C type (Table 4). Nut age or endosperm types did not influence the breadths of embryos, however, increasing trend was also noticed.

Percentage and rate of germination

Percent germination of embryos of 9, 10 and 11-month old nuts were found to be statistically similar (Figure 3 A). Apparently, as early as the 9th month, the embryo of Makapuno nut is already fully developed, mature and capable of germination. On the other hand, rate of germination, evaluated on the basis of number of days to germination (appearance of the first scale leaf) was inversely related to the fruit age (Figure 3 B). Similar trend of germination was obtained in embryos of A, B and C type endosperm (Figure 4 A and B). Although, the 9-month-old embryos are slow to bud break, but after germination, the rate of growth of plantlet is similar to the other types. It appears that the types of endosperm are related to the nut age. Embryos of A type nut still need some more time to initiate germination. The result implies that A, B and C type nuts are equivalent to the ages of 9, 10 and 11 months old nuts, respectively. It is interesting to note that 9-12 months old embryos of normal nuts seem to perform equally well in the in vitro propagation (Mkumbo et al., 1998). Karun et al. (2002) also reported the influences of age of embryos on the in vitro growth of plantlet.

Immature embryos are considered physiologically dormant (Gardner et al. 1985). A deficiency of growth promoting substances or the improper hormonal balance could cause dormancy (Krishnamoorthy, 1981).

Rate of germination of embryos of different ages in different growth stages

Embryos were inoculated in liquid medium for initial growth. Enlargement of the embryos were manifested by swelling in girth and protrusion of plumule. Growth rate in first liquid medium was faster and equivalent in embryos of 10 and 11 month old fruits as compared to the embryos of 9 month old. No significant difference was observed in period of germination among the age groups of embryos. Growth of 11 month old nuts was found faster and transferred again to a second liquid medium earlier than 9 and 10 month old embryos (Fig 5). It appeared from the result that embryos of nine month old fruits were physiologically immature and took more time during incubation in the first liquid growth medium to attain maturity before germination. Karun et al., (2002) similarly, reported the influences of age of embryos on the in vitro growth of plantlet.

The correlation coefficients of embryo germination % with fruit, nut and embryo morphology were estimated. Germination of embryo showed significant and positive correlation with embryo weight and nut/fruit weight ratio while the germination % significantly and negatively correlated with husk/nut%. A significant and positive correlation was observed between length and breadth of embryos (Table 5).

Morphology of in vitro plantlets

In vitro growth and development of transferable plantlets of embryos of 9, 10 and 11 months old nuts were found similar with respect to plant height and leaf numbers. Numbers of roots of plantlets were fund to be influenced by nut age. Plantlets of 11 months old embryos developed 5.4 roots, which were statistically higher than 3.8 and 3.2 roots of 10 and 9 months old embryos, respectively. Root numbers of 9 and 10 months old embryos were found statistically similar (Table 6). These results corroborated with the findings of Karun et al. (1998) who reported uniformity in leaf production and plant height of in vitro plantlets of 8, 10 and 11 months old embryos after 10 months of inoculation. Uniformity was also observed in ex vitro seedling growth of embryos of 8, 10 and 11 months old nuts (data not shown).

CONCLUSION

Nut age influences the A, B and C types of Makapuno where A was less mature than B and C. The same was true for embryos of A, B and C type Makapunos, since the 9-month-old embryos were slow to germinate compared to the other types. The excised embryos might have different responses in vitro germination because of variable maturity.

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Table 1. Weight and size of fruit of embryo cultured Makapuno coconut palms grown at UPLB as influenced by harvesting age.

AGE (Month)	FRUIT WEIGHT (kg)	HUSK WEIGHT (kg)	POLAR DIAMETER (cm)	EQUATORIAL DIAMETER (cm)	POLAR CIRCUM-FERENCE (cm)	EQUATORIAL CIRCUM-FERENCE (cm)
9	1.74	0.78	17.35	16.41	54.68	53.80
10	1.54	0.64	17.45	16.86	59.38	53.48
11	1.56	0.68	17.50	16.45	54.38	53.51
CV (%)	22.89	43.14	11.70	9.47	8.58	8.65
Level of significance	ns	ns	ns	ns	ns	ns

ns= not significant

Table 2. Weight and size of nut of embryo cultured Makapuno coconut palms grown at UPLB as influenced by harvesting age

AGE	WEIGH	POLAR LENGTH	EQUATORIAL LENGTH	POLAR CIRCUMFERENCE	EQUATORIAL CIRCUMFERENCE
(Month)	(kg)	(CM)	(cm)	(cm)	(cm)
9	0.96	11.46	12.54	38.17	40.24
10	0.91	11.87	13.01	38.45	40.35
11	0.91	11.62	12.83	37.87	40.51
CV (%)	22.05	12.34	9.38	5.82	6.17
Level of significance	ns	ns	ns	ns	ns

Table 3. Weight, length and breadth of embryos of UPLB Makapuno coconut population as influenced by harvesting age of fruit

NUT AGE	WEIGHT	LENGTH	BREADTH
(Month)	(g)	(cm)	(cm)
9	0.16	0.73 b	0.50
10	0.17	0.87 a	0.51
11	0.19	0.96 a	0.56
CV (%)	40.24	16.28	19.99
Level of significance	ns	**	ns

ns = not significant, ** = significant at 1% level of probability, means having same letter in a column are not significant at 5% probability)

Table 4. Weight, length and breadth of embryos of UPLB Makapuno coconut population as influenced by types of endosperm

TYPE	WEIGHT (g)	LENGTH (cm)	BREADTH (cm)
A	0.16 c	0.79	0.39
B	0.21 b	0.84	0.39
C	0.26 a	0.87	0.43
Level of significance	**	ns	ns
CV(%)	8.36	4.49	11.02

** = significant at 1% probability; means having same letter in a column are not significant at 5% probability, ns = not significant

Table 5. Effect of different phenotypic characters on germination

	Germination (%)	Embryo	Embryo	Embryo	Nut	Fruit	Husk	Husk/nut	Nut/fruit
Germination (%)	X								
Embryo weight (g(	0.86**	X							
Embryo length (cm)	0.38	0.80	X						
Embryo breadth (cm)	-0.10	0.42	0.88**	X					
Nut weight (g)	-0.71	-0.97	-0.92	-0.63	X				
Fruit weight (g)	-0.77	-0.99	-0.88	-0.56	1.00	X			
Husk weight (g)	-0.88**	-01.00	-0.78	-0.39	0.96	0.98	X		
Husk/nut (% w/w)	-0.98**	-0.95	-0.57	-0.11	0.84	0.98	0.96	X	
						-			
Nut/fruit (% w/w)	0.98**	0.96	0.57	0.11	-0.84		-0.96	-1.0	X

** = significant at 1% level of probability
* = significant at 1% level of probability

Table 6 Influence of nut age on seedlings growth

NUT AGE (Month)	HEIGHT OF SEEDLING	LEAF NUMBER	COLLAR DIAMETER OF SEEDLING	ROOT NUMBER
9	41.1	4.1	1.1	3.2 b
10	41.3	3.8	1.2	3.8 ab
11	41.5	3.9	1.1	5.4 a
CV (%)	14.53	21.01	15.83	41.70
Level of significance	ns	ns	Ns	*

Values within a column followed by the same letter(s) are not significantly different by DMRT (p = 0.05)

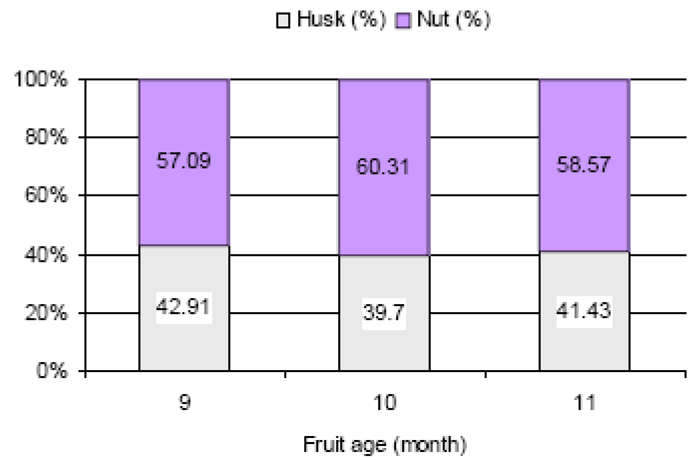


Figure 1. Ratio between fruit component as influenced by harvesting age of Makapuno

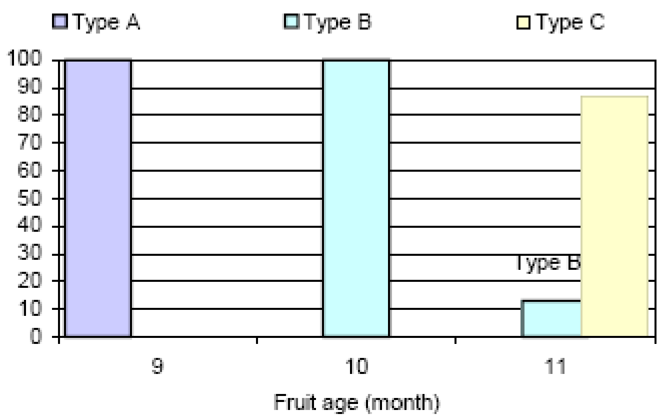
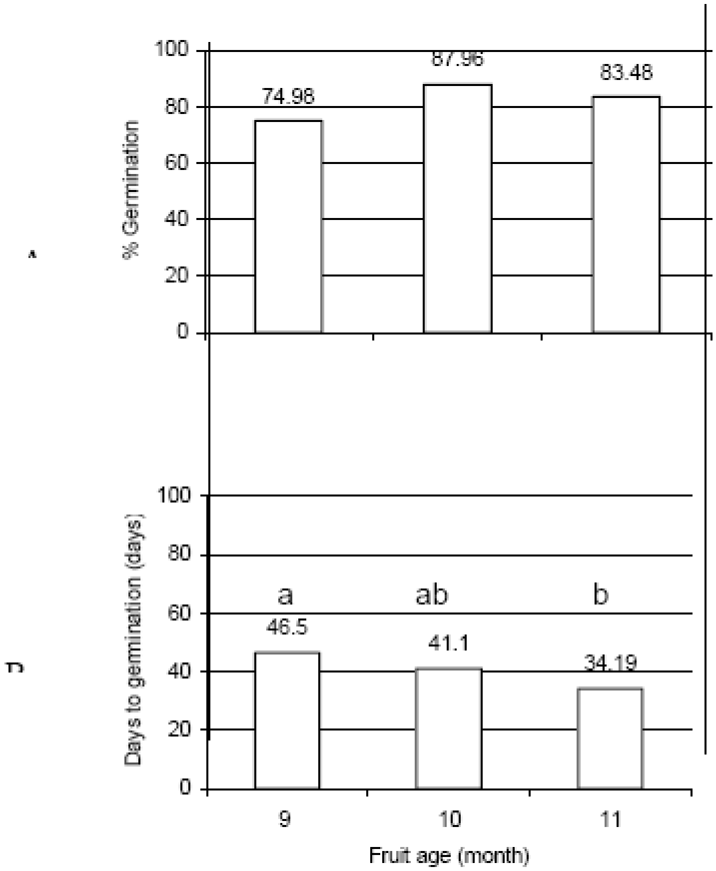
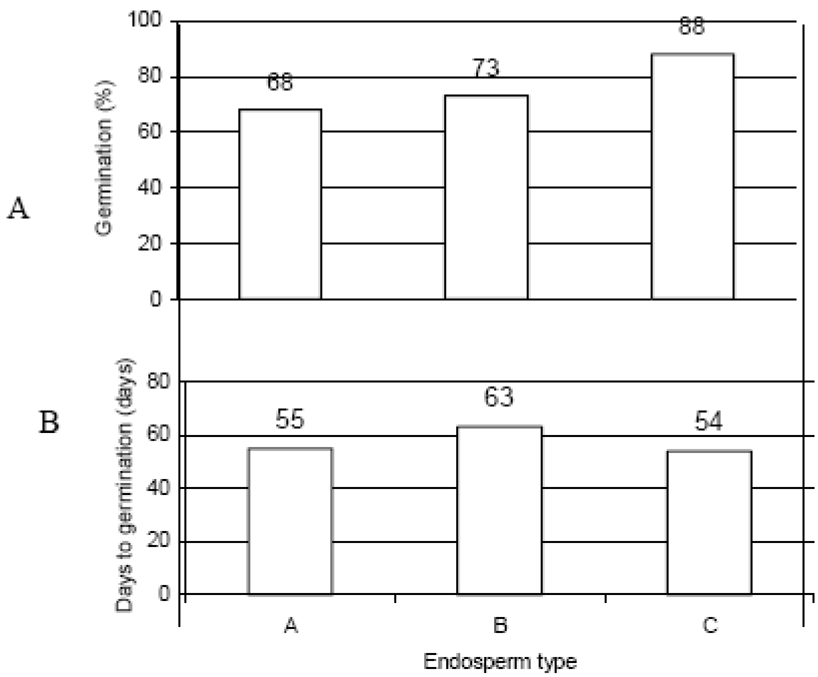


Figure 2. Effect of nut age on expression of A, B and C types Makapuno



Days to germination was counted from date of first inoculation in liquid medium

Figure 3. Percent germination and days to germination of embryos of fruits of UPLB Makapuno coconut palms harvested at 9, 10 and 11 months



(Days to germination was counted from the date of first inoculation date in liquid medium)

Figure 4. Percent germination and days to germination of embryos excised from A, B and C types endosperm

(Columns followed by same letters are not significantly different ot 5% level ofprobability)

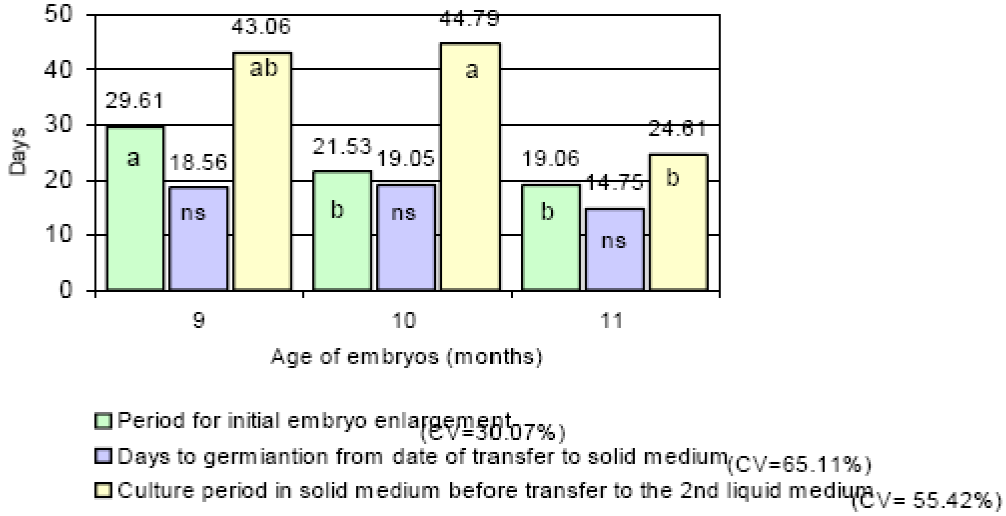


Figure 5. Growth of embryos and plantlets in different stages of culture