

Research Note: Polyembryony in Philippine Makapuno (*Cocos nucifera* L.) Hybrids

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Abstract

Polyembryony was found in five Philippine makapuno hybrids developed by the Visayas State University (VSU) namely: VMAC1, VMAC2, VMAC3, VMAC4 and VMAC5. Different numbers of multiple embryos in a nut, such as the most commonly encountered twins (2), the rare triplets (3) and quintuplets (5), were observed at very low frequencies of 0.7-10% per hybrid during some harvest periods. The multiple embryos were found clustered together under the 'soft eye' of the nut but they did not share any tissue.

Among the hybrids, VMAC1 had the most frequent occurrence of polyembryony. Multiple embryos were encountered in VMAC1 in majority of the harvest periods. In the other four hybrids, polyembryony was very rarely observed.

In vitro germination and development of the cultured polyembryos was almost synchronized. All polyembryos developed normally *in vitro*.

Keywords: Polyembryony, coconut, *Cocos nucifera*, makapuno hybrids, *in vitro* growth.

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Introduction

Polyembryony is the development of two or more embryos in one seed or the development of more than one embryo from a single egg or ovule. Extra embryos are produced in two ways. Commonly, the formation of multiple embryos was found from cells of the seed parent (nucellar embryony) as in the case of *Coelebotryne* (Euphorbiaceae), *Funkia* (Liliaceae) and a species of *Allium*, while in some other cases, any cell within the embryo-sac may exceptionally form an embryo, e.g. the synergidae in *Mimosa* species and antipodal cells in *Iris* and *Allium* (<http://www.fruitsinfo.com/Fruit-development.htm>). Occasionally, polyembryony may develop by the production of two or more zygotic embryos either by fission of one fertilized egg or splitting of one embryo into several embryos called cleavage polyembryony, or from two or more functional embryo sacs in a single ovule (Kuug and Bacchi, 1943). This is also known as zygotic polyembryony which is found in higher plants like mandarin orange (Das, et. al., 2007). In addition, the fertilized egg of some plants may form a mass of tissue on which several embryos are produced.

Rare cases of polyembryony have been reported in coconut where two to three shoots were observed to emerge from the nut, each through a separate eye or all through the soft eye (<http://www.ikisan.com>, <http://www.bioiversityinternational.org>, Arunachalam, 2005). The occurrence and *in vitro* development of multiple embryos in five Philippine makapuno (an aberrant form of coconut with thick, soft and glutinous meat endosperm which almost fills the nut) hybrids developed by the Visayas State University (VSU) in the City of Baybay, Philippines is hereby reported. The hybrids were developed through controlled pollination using four dwarf coconut cultivars namely, Coconino, Malayan Red Dwarf, Malayan Yellow Dwarf and Tacunan as maternal parents. Pollen sources were the tall homozygous makapuno palms of the University of the

Philippines at Los Baños (UPLB), College, Philippines.

Methodology

Polyembryony was recorded while embryos from the VSU makapuno hybrids namely, VMAC1 (Coconino x UPLB Makapuno), VMAC2 (Malayan Red Dwarf x UPLB Makapuno), VMAC3 (Malayan Yellow Dwarf x UPLB Makapuno, dwarf type), VMAC4 (Malayan Yellow Dwarf x UPLB Makapuno, tall type) and VMAC5 (Tacunan x UPLB Makapuno), were extracted from meat cylinders of 10-11 month old nuts for *in vitro* culture. Since Philippine homozygous makapuno nuts do not germinate, embryo rescue must be done to grow the F2 and F3 makapuno hybrids. During embryo extraction, the number of embryos per nut was recorded. The frequency of nuts with a certain type of polyembryony was determined in each hybrid per harvest during the two-year observation period from July 2006-June 2008.

Polyembryos from a nut were inoculated together in a single culture bottle with VSU modified Y3 (mY3) culture medium (Nuñez, 2007). The modified medium has the components of Y3 (Eeuwens, 1976) except for the vitamin components which were modified by VSU. During the initial culture, the solid form of mY3 was used as germination medium. Subsequent cultures had the liquid form of the medium. Their development *in vitro* was monitored but no comparison with the single embryos was done.

Results and Discussion

Frequencies and forms of polyembryony in five Philippine makapuno hybrids

The occurrence of multiple embryos was observed in 10-11 month old nuts of VMAC1, VMAC2, VMAC3, VMAC4 and VMAC5. Most of the observed polyembryos were twins. There was a case of triplets and another case of quintuplets but no quadruplets were encountered (Table 1). Frequency of polyembryony per

Table 1. Frequencies and forms of polyembryony in five Philippine makapuno hybrids

Date of harvest	Makapuno Hybrid Cross	Number of nuts	Number of nuts with polyembryos	% polyembryony	Type of polyembryony
July 06	VMAC2	55	1	1.8	Quintuplets
August 06			0		
September 06			0		
October 06	VMAC1	495	8	1.6	Twins
November 06	VMAC1	243	3	1.2	Twins
	VMAC2	64	1	1.6	Twins
December 06			0		
January 07	VMAC1	569	12	2.1	Twins
February 07	VMAC1	141	1	0.7	Twins
	VMAC3	39	3	7.7	Twins
March 07	VMAC4	10	1	10.0	Twins
April 07			0		
May 07			0		
June 07			0		
July 07	VMAC1	85	2	2.4	Twins
	VMAC2	38	1	2.6	Twins
August 07	VMAC1	104	1	1.0	Twins
			1	1.0	Triplets
September 07	VMAC1	209	5	2.4	Twins
October 07	VMAC1	126	4	3.2	Twins
November 07			0		
December 07	VMAC5	92	1	1.1	Twins
January 08	VMAC1	98	1	1.0	Twins
February 08	VMAC1	116	4	3.4	Twins
March 08	VMAC1	64	1	1.6	Twins
April 08	VMAC1	147	3	2.0	Twins
May 08	VMAC1	250	10	4.0	Twins
June 08	VMAC1	280	7	2.0	Twins
	VMAC4	30	1	3.0	Twins

Figure 1. Twin embryos in a meat cylinder of makapuno hybrid VMAC1

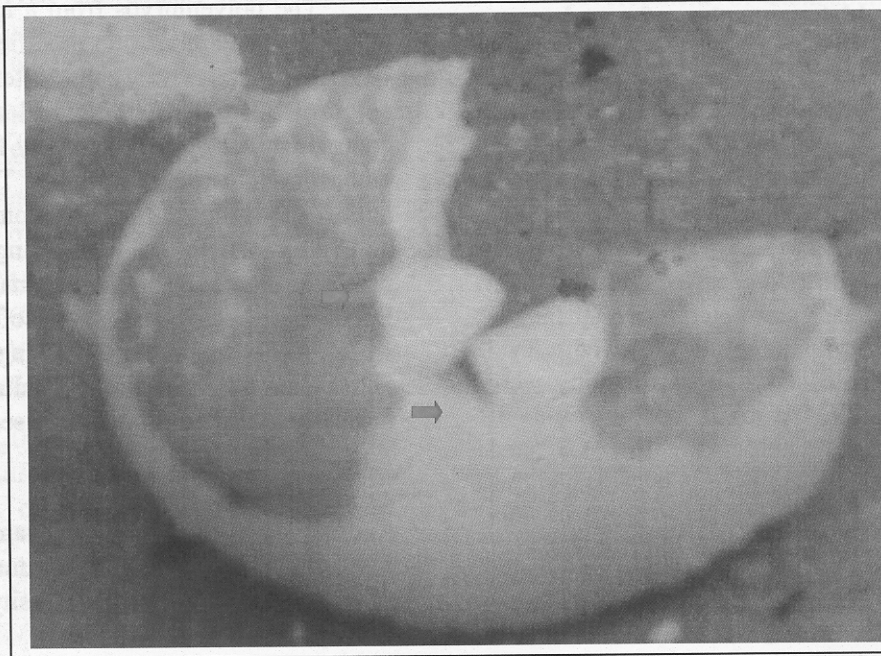


Figure 2. One-month-old twin embryos of VMAC 1 with synchronized germination of twins



Figure 3. Twin embryos of VMAC1 with similar rate of development of shoots



harvest ranged from 0-4.0% in VMAC1, 0-2.6% in VMAC2, 0-7.7% in VMAC3, 0-10% in VMAC4 and 0-1.1% in VMAC5. VMAC1, which had the highest number of sample nuts, had the most frequent occurrence of polyembryony among the five hybrids. Multiple embryos were recorded in this hybrid in 14 out of 17 harvest periods when polyembryony was present. Most cases of polyembryony in VMAC1 were twins. In VMAC2, the occurrence of multiple embryos was recorded in three harvest periods with two cases of twins and one case of quintuplets. Quintuplets were so far the highest form of polyembryony observed. Two cases of multiple embryo formation were encountered in VMAC4. A single case of polyembryony was noted in VMAC3 and VMAC5. VMAC3 and VMAC4 hybrids had few sample nuts per harvest but percentages of nuts with twin embryos were quite high at 7.7% in VMAC3 and 3% to 10.0% in VMAC4.

The cause of polyembryony in the reported Philippine makapuno hybrids has not yet been studied but a possible factor is the presence of meiotic abnormalities which were observed among the maternal parents of the hybrids (Nuñez, 1986). In all nuts with polyembryos, the multiple embryos were clustered together in the depressed portion of the meat endosperm under the 'soft eye' of the nut where normally single embryos were supposedly located. Although closely adherent, the multiple embryos observed were fully separated from each other (Figure 1). They did not share any tissue as in the case of zygotic cleavage polyembryos observed in some crops like mandarin orange where the zygotic polyembryos develop by fission of the original zygotic embryo. It was observed that polyembryos of mandarin orange shared cotyledons. Some twins also had a common primary root; some had common hypocotyls and primary root while others had fused hypocotyls and epicotyls (Das, et. al., 2007). Therefore, it is more likely that the observed multiple embryos among the VSU makapuno hybrids developed from separate egg cells or by fission of the fertilized egg and not by cleavage of the zygotic embryos.

Development of the makapuno polyembryos *in vitro*

The polyembryos from a nut germinated simultaneously *in vitro* in modified Y3 medium beginning at two weeks after inoculation (Figure 2). Each embryo had a shoot and a root which developed separately from the other embryos from the same nut. They also had almost the same rate of development of shoots and roots (Figure 3). Interestingly, all of the polyembryos germinated normally *in vitro*. Polyembryony may be very useful in coconut where the production of planting materials of elite types like makapuno is limited since a nut normally gives rise to only one seedling.

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