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Doubling macapuno seedling production through embryo splitting

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ABSTRACT

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A simple means of deriving two seedlings from a hybrid macapuno embryo was developed. Macapuno embryos were allowed to grow *in vitro* for 1 to 1.5 months or until the shoots and roots emerged. Germinating embryos were cut longitudinally, equally dividing the shoots and roots. Split embryos were then subcultured in an appropriate medium until seedlings were fully developed and ready for potting.

During the first month in culture, 82% of the embryo halves developed normal shoots and roots. After the first month, however, only 2 pairs of split embryos had both halves developing normally. Others had only one of the halves producing a complete plantlet while the other pair died.

The first potted seedling grown from a halved embryo had 5 leaves and 5 primary and adventitious roots with plenty of root hairs during potting at 7.5 months after initial culture. It was 22 cm long with a base diameter of 1.4 cm.

Keywords: coconut. macapuno. embryo culture. embryo splitting.

INTRODUCTION

New macapuno hybrid types which proved to be precocious and high yielding were developed at the Regional Coconut Research Center (RCRC) of the Visayas State College of Agriculture (ViSCA), Baybay, Leyte. These

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palms are F_2s but F_3s are also being grown. Some of these are already planted in the field. Although not expected to be the same as the F_2s in some characteristics, F_3s potential to yield 100% macapuno and their high degree of selfing are expected to be inherited. Thus, planting materials of both F_2s and F_3s are being produced *in vitro* but in limited number due to the limited sources of hybrid embryos. Normally, only one seedling could be grown from an embryo, thus, limited number of seedlings could be obtained from the few existing macapuno-bearing hybrid trees at ViSCA.

A lot of resources have now been spent on the development of techniques for the clonal propagation of coconuts (*Cocos nucifera* L.) that could pave the way for the rapid propagation of elite coconut palms. Development of such technique is possible and in fact there have been reports of successful plant regeneration from vegetative coconut tissues that could yield true-to-type seedlings (Rillo, 1989). However, such successes are not yet highly repeatable. Than-Tuyen and Apurillo (1992) successfully developed a procedure for callus induction, somatic embryogenesis and plant regeneration from cultured zygotic embryos of normal coconut but needs refinement before it can be applied to macapuno. A well-established and highly reproducible procedure for *in vitro* rapid propagation of macapuno is still nonexistent. Hence, a simple means of deriving more than one seedlings from a macapuno embryo through splitting was explored.

MATERIALS AND METHODS

To test the feasibility of producing more than one seedlings from a macapuno embryo through splitting, four 10-month old embryos from a bunch harvested from a tall pure macapuno palm at the University of the Philippines Los Baños, Laguna (UPLB) were used in a preliminary experiment. Embryos were excised from the nuts, sterilized with 5.25% sodium hypochlorite solution and washed with sterile water before cutting. One embryo was halved, another one was cut into 3 equal parts, a third one was quartered while a fourth was planted whole and served as the control. Cutting was done longitudinally. One piece was planted per culture tube using the RCRC modified Y_3 medium of Euwens (1978) with higher iron and sucrose concentrations. Growth of each seedpiece *in vitro* was monitored.

For the second trial, 10-month old macapuno embryos were obtained from the hybrid trees CÑO3, CÑO8, M51, M52, M61, M66 and MS17. Embryos were sterilized after extraction. Instead of cutting the embryos immediately after excision from the nut, they were allowed to grow *in vitro* in the modified Y_3 medium until the shoot and root emerged following the established procedure for embryo culture of coconut (de Guzman *et al.*, 1978; Rillo, 1995). Each germinating embryo was then halved, equally dividing the developing and embryonic root. Split embryos were planted in fresh modified Y_3 medium, one half embryo to a culture tube and kept in a dark chamber for 2-6 weeks. They were then exposed to flourescent light for 16 hours per day until the 4-5 leaf stage was attained.

RESULTS AND DISCUSSION

Splitting before germination

Initial macapuno embryo splitting work showed that simple slicing could yield only a maximum of 2 seedlings due to the characteristic growth of the embryos. The shoot and the embyonic root grow almost opposite each other on the flat portion of the embryo. Thus, only one slice cutting through both the shoot and the embryonic root could be done.

One of the split embryos which were rightly cut by chance developed into a complete seedling *in vitro* but did not survive when potted. The other half was contaminated before germination. Shoot and root emergence in the split embryo was faster than in the whole embryo. Thirty days after planting, the split embryo produced 8 mm shoot and 10 mm roots while the whole embryo had no shoot and root growth yet. Embryos cut into 3 and 4 pieces developed incomplete plantlets. Some had shoots but with no roots while others had roots without shoots. A few just increased in size with spongy growth.

Age of embryos suitable for splitting

After the preliminary trial, macapuno embryos were first germinated *in vitro* before splitting. The germinating embryos were as young as 29 to 120

Table 1. Growth stages of macapuno embryos at splitting and 2-4 weeks later

Source of Embryo	Age in culture at splitting (days)	Shoot/Root Length (mm) At splitting		2 wks after splitting	4 wks after splitting	Remarks
CÑO3	101	4/<1	a	8/5	25/25	
			b	10/4	16/3	no shoot meristem
CÑO8	108	2/<1	a	3/no root	4/no root	
			b	3/no root	7/no root	
M51-1	65	2/<1	a	2/4	20/4	
			b	8/2	10/2	
M51-2	32	3/2	a	5/3	16/2	
			b	contaminated	-	
M51-3	42	3/4	a	13/dead	28/2	
			b	6/dead	12/2	
M51-4	91	9/4	a	5/4	23/10	
			b	11/9	23/25	
M52-1	120	7/6	a	12/7	15/7	
			b	12/4	14/4	
M61-1	48	6/9	a	18/25	23/28	
			b	11/16	15/16	
M61-2	48	5/8	a	10/12	23/12	
			b	6/10	11/10	
M61–4	84	7/8	a	20/15	20/15	
			b	5/8	18/8	
M61-5	42	2/2	a	5/2	9/3	
			b	6/8	15/10	
M61-6	29	1/1	a	7/5	16/21	
			b	6/4	18/17	
M66	38	<1/<1	a	2/1	15/15	
			b	2/1	16/6	
MS17-1	101	<1/<1	a	2/7	7/45	no shoot meristem
			b	5/3	15/28	

Good shoot/root development of embryo halves: 82%

days after initial culture. Age varied much due to differences in the rate of germination of the embryos (Table 1). Some embryos took much longer time to germinate than others. Other embryos were allowed to grow longer shoots and roots before being split so that the effect of the varying growth stages on the growth of the resulting half embryo after splitting could be observed.

Most often, germinating embryos in the modified Y_3 medium had about 1 mm to 3 mm shoot and root length one month after culture. At this age, splitting of an embryo into two almost equal parts is easier than at later stages of development when the shoots and roots are longer. Of the 14 embryos used in the second trial, 2 were not successfully cut at the shoot meristems as evidenced by the absence of the growing point one month after cutting (Table 1). Good splitting resulted in almost equal growth of the 2 seedlings derived from a single embryo (Fig. 1). With longer shoots and roots, unequal division of the growing point is more likely to happen and may result in a normal shoot & root growth of one half of the embryo while the other half may later die due to the absence of the growing point of the shoot (Table 2 and Fig. 2).

Table 2. Development of split hybrid macapuno embryos after 1 month *in vitro*.

Split hybrid macapuno embryos	Development	Percentage*	No. of Seedlings	
			Expected	Actual
M51-1, M51-2, M51-3, M51-4, M52-1, M61-1, M61-2, M61-5, M61-6	Shoot meristem of one of the halves from an embryo died 1.5-4 mos. after subculture	75	18	9
CÑO8	Good shoot but poor root development	8	2	2
M61-4 and M66	Both halves from an embryo developed well	17	4	4
TOTAL			24	15

* Proportion of the 12 embryos with split shoot meristems.

Fig. 1. Halved embryos of M66 with almost equal growth.

Fig. 2. Unequal growth of split embryos due to unequal division of the growth point of the shoot.

Nevertheless, improved skill in cutting germinating embryos would significantly lessen unequal division of the shoots.

It appears that the growth of embryos after splitting was not affected much by its size before splitting. One month after subculture, germinating seedlings from split embryos with less than 1 mm to 3 mm shoots when sliced were almost of the same size as those developing from embryos with 6-9 mm shoots when split (Table 1).

Growth and development of halved hybrid macapuno embryos in vitro

Splitting macapuno embryos into halves was not detrimental to the growing young plant. Split embryos continued to grow, most often normally, *in vitro*. Shoot and root lengths were almost doubled 2 weeks after slicing. Growing seedlings were exposed to light 2-6 weeks after incubation in the dark. Four weeks after splitting, shoots were 4-28 mm long and roots were either poorly or well-developed.

Of the 12 embryos with sliced shoot meristems, 75% were apparently unequally cut (Table 2). Sliced pieces with presumably bigger shoot meristem portions produced bent shoots with either poor or good root development. Those with smaller shoot meristem portions grew normally for a while but after 1.5 to 4 months upon subculture, their shoot meristem died while roots grew vigorously. Vigorous root growth was also believed to be the cause of stunting or death of the developing shoot in an earlier study by Ashbuner *et al.* (1995).

Unequal division of the roots occurred more often but it was not as critical as that of the shoot. Since roots frequently bend during development, cutting them equally was more difficult than cutting shoots. However, unequally divided roots still developed normally especially when cultured in modified Y_3 with higher level (5.5%) of sucrose. Secondary roots grew faster when growing tips of the primary roots were cut off.

One of the halved embryos of CÑO8, developed normal shoot but its root development was very poor possibly due to innate abnormality. Good development of both 'halves' was observed only in M61-4 and M66 or 17% of the embryos with split meristems possibly due to almost equal division of the shoot meristems. Although growing shoots tended to bend toward the cut

portion during the early stage of growth, they eventually grew straight later. Fungal contamination, however, eliminated half of each embryo. Surviving halves were already taken out to the screen house for hardening/potting. M66 b was potted at 7.5 months after initial culture with 5 leaves and 5 primary adventitious roots with plenty of root hairs. It was 22 cm long with 1.4 cm base diameter at potting. Likewise, M61-4 b was very robust and ready for potting.

Except for about 1.5 to 3.5 months extension in incubation period, the older batches of seedlings that grew from halved embryos did not exhibit detrimental effects of cutting.

The technique of producing two seedlings from a macapuno embryo developed by ViSCA is far from perfection yet. A lot of things need to be improved. The most critical is the skill in equally dividing the shoot meristem which could be greatly improved by practice. Another is the reduction of contamination during the incubation period as well as the possible modification of the culture medium to equally favor the development of both the halved shoot meristem and root. Inspite of a number of difficulties, it was demonstrated that producing two genetically identical seedlings from a macapuno or coconut embryo is possible. Once this procedure is perfected macapuno seedling production from embryos could then be doubled.

REFERENCES

ASHBUNER, G. R., M. G. FAURE, D. R. TOMLISON and W. K. THOMPSON. 1995. A guide to the zygotic embryo culture of coconut palms (*Cocos nucifera* L.) *Technical Reports* No. 36, ACIAR, Australia.

DE GUZMAN, E.V., A.G. DEL ROSARIO and E.M. UBALDE. 1978. Proliferation, growth and organogenesis in coconut embryo and tissue cultures. *Philippine Journal of Coconut Studies* **3**: 1-10.

EUWENS, C.J. 1978. Effects of organic nutrients and hormones on growth and development of tissue explants from coconut (*Cocos nucifera* L.) and date (*Phoenix dactylifera*) palms cultured in vitro. *Physiological Plantarum* **42**: 173-178.

RILLO, E.P. 1989. Coconut Tissue Culture. A tool for improved coco production. *Coconuts Today*. pp. 113-122.

RILLO, E. P. 1995. *Embryo Culture of Coconut*. A Laboratory Manual. (In Press).

THAN-TUYEN, N.T. and D.I. APURILLO. 1992. Plant regeneration through somatic embryogenesis from cultured zygotic embryos of coconut. *Philippine Journal of Coconut Studies* **17**: 7-11.