PHILIPPINE "GULAMAN" AS SUBSTITUTE OF IMPORTED AGAR IN CULTURE MEDIUM FOR COCONUT EMBRYOS[1]

N.T. Thanh-Tuyen and D.I. Apurillo[2]

Substitution of imported agar (Maru agar, Japan) by locally available food-grade agar (gulaman, Philippines) was tried in embryo culture medium for *in vitro* germination of three coconut cultivars, namely, Baybay Tall, Coconiño, and Lingkuranay. There was no significant difference in the efficacy of gulaman and Maru agar which were used at the same concentration in three sequential semi-solid media for embryo germination.

In vitro culture of coconut embryos was first reported by Cutter and Wilson (1954) with the elucidation on the role of the endosperm during the post-embryonic growth of the embryo. Subsequent researches on coconut embryo culture succeeded in including germination and seedling development on artificial media (Abraham and Thomas 1962; de Guzman and del Rosario 1964; Ventura et al. 1966).

The detailed technique of *in vitro* culture of coconut was then developed by de Guzman and associates (de Guzman 1969; Balaga and de Guzman 1971; de Guzman et al. 1971; del Rosario and de Guzman 1976), who pioneered in makapuno embryo culture to overcome the problem of non-germination of the "abnormal" coconut under natural conditions. The success has led to the commercialization of makapuno seedlings in the Philippines.

Due to the bulk of coconuts and inadvertent introduction of diseases harbored in the seednuts, *in vitro* culture of coconut embryos has been recognized as a valuable tool in expedition and conservation of coconut germplasm (de Guzman 1979; Withers 1987, Sossou et al. 1987; Assy Bah et al. 1987). After all, the weight of one seednut is equivalent to 10,000 embryos (cf. de Guzman 1979).

With the above-mentioned applications of *in vitro* culture of coconut embryos, any measures which could reduce the production cost per seedling would be highly desired. In this context, substitution of imported agar by locally available food-grade agar, gulaman, without causing adverse effects on germination of the embryo and development of the seedling, is one of the possible cost-savings measures. Such substitution is taken into consideration because of the fact that the gelling agent is usually consumed in larger quantity than other ingredients in the culture medium.

The embryos were excised from mature nuts (ca.10-11 mo) of three coconut cultivars; namely Baybay Tall, Coconiño, and Lingkuranay.

The techniques of embryo excision, surface sterilization and inoculation, the sequence of transfers of the cultured embryos from liquid medium to three successive semi-solid media, and the medium formula were adopted from de Guzman and del Rosario (1964), Balaga and de Guzman (1971), and del Rosario and de Guzman (1976).

In the semi-solid media, 0.8% (w/v) Maru agar or gulaman were used for gelling purpose. The preparation of gulaman for gelling the culture medium involved washing with water, soaking in 70% ethanol, air-drying until the gulaman reached the original dryness, and weighing (T. Murashige 1984, personal communication). The agar and gulaman were cooked in a microwave oven before they were mixed with other ingredients. The culture medium was adjusted to pH 5.8, then autoclaved.

The evaluation on the efficacy of Maru agar and gulaman as the gelling agent was reckoned as percent cultured embryos with balanced and good shoot and root development. The rating was carried out following the standards shown in Figures 1-3.

After weeks in the initial liquid medium, the cultured embryos with the emerging shoot and root apices were transferred to the first semi-solid medium for shoot and root elongation. The seedlings were then transferred every 8 wk to the second and third semi-solid media for further development. The three media had varied compositions as needed for different stages of seedling development.

It was noted that the primary roots and scale leaves of the coconut seedlings developed in the first medium. Initiation of secondary roots and development of opened leaves were observed in the second medium. In the third semi-solid medium, the root system proliferated and the seedlings generally began to have split leaves. The vigorous seedlings with profused root systems could be transferred to pots for establishment in soil. Otherwise, the seedlings were maintained for another 8-wk period on the third semi-solid medium before establishment in pots.

Table 1 presents the performance of the coconut embryos cultured on three successive semi-solid media gelled with either Maru agar or Philippine gulaman. Statistical analysis of the experiment set up split plot arranged in randomized complete block design with three cultivars as main plots and two gelling agents as sub-plots

---

[1]Paper presented at the First National Symposium of Plant Tissue Culture in Philippine Agriculture and Forestry, May 26-28, 1988, U.P. Los Baños. College, Laguna, Philippines.

[2]Tissue Culture Laboratory, Visayas State College of Agriculture (ViSCA), Baybay, Leyte 6521-A, PHILIPPINES.

TABLE 1. Percent cultures of coconut embryos/seedlings with balanced and good shoot and root development evaluated after 8 wk in the first, second and third semi-solid media gelled with Maru agar or gulaman at 0.8% concentration.

Gelling Agent	% Cultures with Balanced and Good Shoot and Root Development*		
	Baybay Tall	Coconiño	Lingkuranay
First medium			
Maru agar	34.7 ns	60.9 ns	59.5 ns
Gulaman	36.8	55.5	64.8
	cv=21.9%		
Second medium			
Maru	55.8 ns	56.1 ns	70.1 ns
Gulaman	37.9	74.5	69.5
	cv-31.86%		
Third medium			
Maru agar	37.5 ns	54.8 ns	61.4 ns
Gulaman	32	58.8	62.9
	cv=24.2%		

*Average of three replicates with 10 samples per replicate.

showed no significant difference in the efficacy of Maru agar and gulaman.

The common apprehension of the use of food-grade agar is the presence in the product of impurities which are unknown and may have adverse effects on the development of *in vitro* cultures. The reported observations indicate that with proper technique of preparation, the cheap Philippine gulaman, can be used with equal efficacy to Maru agar, for gelling the culture medium for *in vitro* germination of coconut embryos, in particular, and for propagation of other crops, in general.

With the price of gulaman being generally about one-fourth of that of Maru agar (the retail prices of Maru agar and gulaman in 1987 were P1,640/kg and P400/kg, respectively), and the fact that Philippine gulaman is locally available, the substitution of imported agar by Philippine gulaman would reduce considerably the production cost per coconut seedling, and generally, the operation expenses for commercial tissue culture systems.

REFERENCES

ABRAHAMS, A. and K.J. THOMAS. 1962. A note on the *in vitro* culture of excised coconut embryos. Indian Coconut J. 15:84-87.

ASSY BAH, B.T. DURAND-GASSELIN, and C. PANNETIER. 1987. Uses of zygotic embryo culture to collect germplasm of coconut (*Cocos nucifera* L.) FAO/IBPGR Plant Genetic Resources Newsl. 71:4-10

BALAGA, H.Y. and E.V. DE GUZMAN. 1971. The growth and development of coconut "makapuno" embryos *in vitro*. II. Increased root incidence and growth in response to media composition and to sequential culture from liquid to solid medium. Phil. Agric. 53:551-565.

CUTTER, VM. and K.S. WILSON. 1954 Effect of coconut endosperm and other growth stimulants upon the development *in vitro* of embryos of *Cocos nucifera*. Bot. Gaz. 115:224-240.

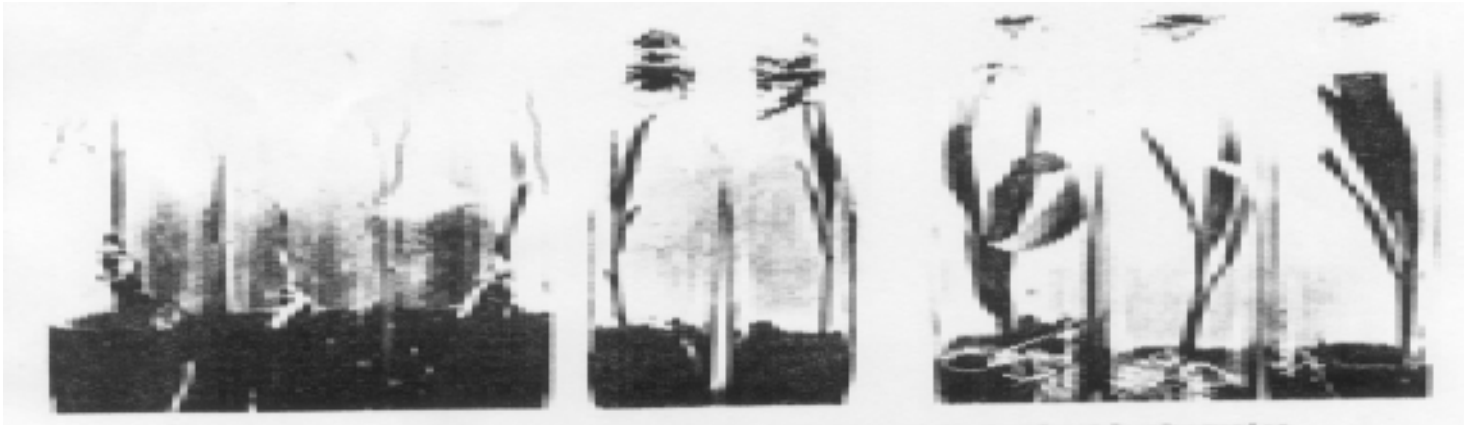
DE GUZMAN, E.V. 1969. The growth and development of coconut "makapuno" embryo *in vitro*. I. The induction of rooting. Phil. Argic 53:65-78.

DE GUZMAN, E.V. 1979. Embryo culture and germplasm exchange. PCRDF Professorial Chair Lecture. July 30, 1979, U P Los Baños, College, Laguna, Philippines.

DE GUZMAN, E.V. and D.A. DEL ROSARIO. 1964. The growth and development of *Cocos nucifera* L. "makapuno" *in vitro*. Phil Agric. 48:82-94.

DE GUZMAN, E.V, A.G. DEL ROSARIO, and E.C. EUSEBIO. 1971. The growth and development of coconut "makapuno" embryo in vitro. III. Resumption of root growth in high sugar media. Phil. Agric. 53:566-579.

DEL ROSARIO, A.G. and E.V. DE GUZMAN. 1976. The growth of coconut "makapuno" embryos *in vitro* as affected by



FIGURES 1, 2, & 3. Coconut seedlings with balanced and good shoot and root development on the first semi-solid medium (1), the second semi-solid medium (2), and the third semi-solid medium (3).

mineral composition and sugar level of the medium during the liquid and solid cultures. Phil. J. Sci 105:251-222.

SOSSOU, J., S. KARUNARATNE, and A. KOVOOR. 1987. Collecting palm: *In vitro* explanting in the field. FAO/IBPGR Plant Genetic Resources Newsl. 69:7-18.

VENTURA, P., L.C. ZUNIGA, and J.E. FIGUERA. 1966. A progress report on the development of coconut embryo in artificial media. Phil. J. Plant Ind. 31:81-87.

WITHERS, L.A. 1987. *In vitro* methods for collecting germplasm in the field. FAO/IBPGR Plant Genetic Resources Newsl. 69:2-6