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Comparative suitability of two culture media to the *in vitro* growth of embryos of three coconut types

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ABSTRACT

Using a suitable medium for specific plant genotype greatly improves the efficiency of the *in vitro* culture method. The Visayas State University (VSU)-based National Coconut Research Center -Visayas (NCRC-V) evaluated the comparative suitability of the COGENT medium and VSU-modified Y3 (mY3) as *in vitro* culture media for coconut embryos using Albuera Dwarf (ALD), Baybay Tall (BAYT),and the VSU-developed Coconiño x Makapuno (VMAC1) hybrid. These two media differ in vitamin components, iron concentration and state during the first two stages of culture, namely germination and first subculture.

Results showed that mY3 was more suitable for the *in vitro* germination and development of the coconut embryos than the COGENT medium. Significantly higher germination rates were observed in BAYT, ALD, and VMAC1 cultured in the semi-solid mY3 than those in the liquid COGENT medium from the first week until the fourth week of initial culture. Germination rates of 100%, 85.8% and 84.5% were obtained from CÑO x MAC, ALD, and BAYT, respectively. Furthermore, significantly higher percentages of germinating embryos with developing shoot and root were observed in the semi-solid mY3 than in the liquid COGENT. Likewise, better growth of plantlets in liquid mY3 was noted during the fourth and fifth months of culture.

Among coconut types, VMAC1 had the highest germination rates in the two media and the best growth in mY3. BAYT had better growth in the COGENT medium while ALD had better shoot development in mY3.

Keywords: coconut, *Cocos nucifera* L., coconut embryo culture, *in vitro* culture media, *in vitro* growth

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INTRODUCTION

Coconut (*Cocos nucifera* L.) is reproduced mainly by seed. Due to the large size of its seed, coconut germplasm collection and exchange is costly and laborious. Hence, the embryo culture technology is considered an efficient, safe, and practical tool for coconut germplasm collection and exchange since it reduces problems in handling and transport of coconut genetic materials. For germplasm exchange, the FAO/IBPGR Technical Guidelines for the Safe Movement of Coconut Germplasm recommends the distribution of coconut as zygotic embryos *in vitro* to reduce chances of introducing diseased material into disease-free areas (Engelmann, 1997; Frison *et al*., 1993). In addition, embryo rescue through *in vitro* culture is the only way to grow the homozygous makapuno (mm) palms.

Makapuno is the most popular and economically important soft-endospermed coconut in the Philippines. However, its production is very limited due primarily to very limited planting materials. Embryos of this aberrant form of coconut do not germinate in the nut due to perishable meat and lack of the enzyme necessary for the mobilization of galactomannans that contribute to germination of their embryos (Rillo, 1997; Samonte, *et al*., 1989) so, embryo rescue and *in vitro* culture must be done.

In the Visayas State University (VSU)-based National Coconut Research Center-Visayas (NCRC-V), breeding work on soft-endospermed makapuno requires the use of the embryo culture technology to grow the homozygous makapuno palms from dwarf x makapuno hybrids. VSU modified Y3 medium (mY3) (Nuñez and de Paz, 1996) is used throughout the culture period with a seedling recovery rate of at least 70%. Considering that different plant genotypes may vary in their responses to different *in vitro* culture medium formulations and in an attempt to improve seedling recovery rate, VSU studied the possibility of using an improved *in vitro* culture medium which was developed by the International Coconut Genetic Resources Network's (COGENT) in collaboration with a number of coconut tissue culturists from France, Vietnam, and the Philippines (Rillo, 2000) for its hybrid makapuno. A ten-month study funded by COGENT was conducted to assess the comparative suitability of the COGENT medium and mY3 on the growth of embryos of three coconut types from the VSU germplasm as well as, to determine genotypic differences in culture responses of the different coconut types to the culture media.

Materials and Methods

Coconut Materials

Three types of coconut namely: Albuera Dwarf (ALD), Baybay Tall (BAYT) and the homozygous makapuno (mm) from Coconiño x Makapuno (VMAC1) hybrid were used as embryo donors. While ALD and BAYT are indigenous to Leyte, Coconiño is a small-seeded self-pollinated dwarf coconut from the University of the Philippines at Los Baños (UPLB). Makapuno pollen used in the development of VMAC1 was also obtained from UPLB but the controlled hybridization work was done in VSU. The embryos were obtained from 10-11 month old nuts of the three coconut types.

Culture media

Two culture media were used in the study; the COGENT medium and the modified Y3 (mY3). The COGENT medium has Y3 (Euwens, 1978) macro and micro nutrients, higher FeEDTA concentration of 4.17 g per liter of medium and the combined vitamins used by UPLB and the Philippine Coconut Authority (PCA)-Albay Research Center (Table 1). Modified Y3 (mY3) also contains the macro and micro components of Y3 but the vitamin components were modified by VSU researchers to suit the requirements of the makapuno hybrid embryos. VSU uses the semi-solid form of the mY3 medium for germination of the hybrid makapuno embryos while other laboratories such as those in UPLB and PCA use the liquid form of COGENT. Fifteen milliliters (ml) of each medium was dispensed to 150 mm x 55 mm test tube for the initial culture. For the final culture, 70 ml of mY3 and 80 ml of COGENT were used. Media preparation was done three days before inoculation of embryos and at least one day before each subculture.

Explant preparation, culture and data gathering

Following the procedure established by PCA's Makapuno Laboratory (Rillo, 2000), coconut embryos were extracted from the makapuno nuts in meat cylinders. Meat cylinders were washed with tap water and surface sterilized using full strength commercial bleach, rinsed thrice with sterile distilled

Table 1. Components (g per liter) of COGENT and mY3 in vitro culture media for coconut embryos

Component	COGENT	mY3
Macronutrients:		
potassium nitrate	20.20	20.20
potassium chloride	14.92	14.92
ammonium chloride	5.35	5.35
sodium phosphate monobasic	3.12	3.12
calcium chloride dihydrate	2.94	2.94
magnesium sulphate heptahydrate	2.47	2.47
Micronutrients:		
manganese sulphate tetrahydrate	1.12	1.12
potassium iodide	0.83	0.83
zinc sulphate heptahydrate	0.72	0.72
boric acid	0.31	0.31
copper sulphate pentahydrate	0.025	0.025
sodium molybdate dehydrate	0.024	0.024
cobalt chloride hexahydrate	0.024	0.024
nickel chloride hexahydrate	0.0024	0.0024
Iron Solution		
sodium EDTA dihydrate	4.17	3.19
Iron sulphate heptahydrate	1.39	1.39
Vitamin Solution		
Thiamine HCl	0.005	0.005
Pyridoxine HCl	0.005	0.005
Nicotinic acid	0.005	0.005
Ca-D Panthothenate	0.005	-
Biotin	0.005	-
Folic acid	0.005	-
Glycine	0.100	-
L-arginine	-	1.00
L-asparagine	-	8.81
L-glutamine	-	10.11
Sugar (Table grade)	60	45
Activated charcoal	1	1
Agar	7	7
pH	5.6	5.6
State of the medium		
Germination	liquid	solid
1st subculture	solid	liquid
2nd subculture	liquid	liquid

water, and inoculated singly on fresh germination medium in test tubes. Forty embryos of each coconut type were used per medium per replication. Three replications were set up.

Inoculated embryos were kept in an air-conditioned incubation room in dark condition. Germination of embryos was monitored weekly. Emergence of either shoot or root primordium of each embryo was considered the start of germination. Length of shoots and/or roots was measured weekly during the first month of culture. After 1 to 1 $^{1}/_{2}$ months in intial culture, germinated embryos with one inch-long shoots were subcultured on fresh media. During the first subculture, roots were cut to approximately one half inch for easy handling and enhancement of the growth of new roots. The number of developing roots was determined at two and three months in culture since it is important in the survival of the seedlings during potting.

RESULTS AND DISCUSSION

In vitro germination of embryos from three coconut types using two culture media

Initial emergence of the shoot was observed in both semi-solid mY3 and the liquid COGENT media. However, higher germination rates were observed from coconut embryos cultured in the semi-solid mY3 than in liquid COGENT medium with BAYT, ALD and VMAC1 having 34.3 %, 38.0% and 39.9% germination, respectively in the solid mY3 one week after inoculation (Fig.1). In the liquid COGENT medium, they had 8.93%, 24.2% and 16.1% germination, respectively. Germination percentages increased rapidly during the second week but slowed down during the third week until the fourth week of observation. After four weeks in culture, BAYT, ALD and VMAC1 had germination percentages of 84.5, 85.8 and 100, respectively.

Among the three coconut types, VMAC1 had the highest germination rates in the two media followed by ALD. BAYT had the least germination percentages in both media. However, differences in their germination rates during the fourth week of culture were not significant.

Across coconut types, mean germination percentages were significantly

figure 1 and 2

higher in semi-solid mY3 than in liquid COGENT from the first week of culture until the fourth week when mean germination reached 90.0% in the former and 63.6% in the latter (Fig.2). This result is in line with the findings of Centro de Investigacion Cientifica de Yucatan (CICY) in Merida, Mexico where higher germination of coconut embryos was observed when gelling agent was added to the germination medium and embryos were inoculated in vertical position with the plumule axis oriented upward (IPGRI, 1998).

The significantly higher germination percentages of the coconut embryos in semi-solid mY3 during the first 4 weeks of culture could not be attributed to a specific factor due to the limitation of the study. However, a study on the effect of embryo positions on embryo germination showed that the vertical position with the plumule end oriented upwards was the best position among other treatments which included horizontal position and vertical position with the plumule axis downwards (IPGRI, 1998). The semi-solid mY3 had an obvious advantage in keeping the embryo in upright position. Developing shoots of germinating embryos in the solid medium were observed to be oriented upward while roots were naturally growing downward (Figs. 3 and 4). In the liquid COGENT, there were three orientations of the growing embryos; shoots were more often oriented horizontally while others were oriented upward or downward. Horizontal orientation was a problem since shoot development was almost always restricted by test tube walls as early as two weeks after inoculation. The embryos enlarged faster in the liquid COGENT even prior to germination. Subculturing to fresh medium must be done before the cultures reach one month to prevent difficulty in taking out the germinating or enlarged embryo from the culture vessel. Downward orientation also posed a problem during transfer since both the shoot and developing root were oriented upward. One possible solution to keep the embryos in the upright position in the liquid germinating medium is the use of paper bridges. However, this has not been tried in this study. It may also entail additional expense, time and labor. Furthermore, coconut embryos do not have flat bottoms and are very slippery thus, keeping them in upright position thru paper bridges may also be difficult.

Shoot and root emergence during germination

The development of both shoot and root is desired in *in vitro* cultured coconut embryos. In this study, the three coconut types showed higher percentage of germinated embryos having developed shoot and root (sr) after

figure 3 and 4

figure 5 and 6

one month of inoculation on the semi-solid mY3 than in the liquid COGENT (Fig. 5). There were few embryos that developed only shoots in the liquid COGENT (so) except for VMAC1 where 39.6% of cultural embryos developed shoots without roots. Fewer embryos in mY3 developed roots (ro); 18% in ALD and 1.0% in VMAC1. Across coconut types, significantly higher percentage of one-month old plantlets with developed shoots and roots was observed in mY3 (Figure 6).

Development of plantlets

One-month old plantlets of BAYT and VMAC1 more often developed longer shoots in the semi-solid mY3 than in COGENT. The opposite trend was observed in ALD (Table 2). The makapuno hybrid VMAC1 had longer roots in the solid mY3. Root development of ALD and BAYT however, did not show a consistent trend. Root length was not measured after the first month since the primary root of a germinated embryo was cut prior to transfer to fresh medium during the first subculture. Number of new roots was then monitored instead of root length during the second and third months of culture. More often, the coconut plantlets in mY3 had more secondary roots. After five months in culture, plantlets of the three coconut types in the liquid mY3 almost always had longer shoots than those in the solid COGENT while roots have tremendously increased in length and numerous tertiary roots have developed so data on their number and length were not gathered.

Moreover, plantlets grown in the former had visibly better developed leaves. Plantlets in mY3 also had better root development than those in COGENT. Generally more secondary roots and root hairs were developed in plantlets cultured in mY3, which had lower iron concentration than COGENT medium. Iron deficiency in the roots is known to decrease root elongation but root diameter and amount of root hairs is increase (DUCHEFA, 1998). Since root hairs are active in water and nutrient absorption, seedlings with more root hairs are believed to have better survival when potted.

Based on shoot and root development, BAYT apparently had the best

Table 2. Mean shoot and root lengths (mm)/root number of developing plantlets of three coconut types cultured in vitro in COGENT and mY3 media

Age (Month)	Culture Medium	Growing Point	ALD	BAYT	CÑO x MAC
1	COGENT	shoot	10.2	9.5	5.5
		root	28.6	16.3	8.9
	mY3	shoot	10.0	11.5	12.6
		root	17.8	16.4	23.6
2[1/]	COGENT	shoot	21.4	20.1	11.7
		root	1, 2	0, 2	1, 2
	mY3	shoot	24.8	25.2	22.9
		root	0, 3	0, 4	1, 2
3[1/]	COGENT	shoot	43.3	30.3	27.0
		root	1, 4	0, 2	1, 7
	mY3	shoot	42.2	42.3	44.6
		root	0, 4	1, 7	1, 7
4[2/]	COGENT	shoot	59.6	67.8	52.7
	mY3	shoot	104.1	90.6	133.0
5[2/]	COGENT	shoot	77.0	95.0	84.7
	mY3	shoot	123.4	108.8	175.0

[1/] Number of additional roots during the first subculture, first number ismean primary root while the second number is mean secondary roots

[2/] Shoot length during the second subculture

growth among the three genotypes in COGENT. VMAC1 had the best growth in mY3 since the medium was developed to suit the need of the makapuno hybrids.

CONCLUSION AND RECOMMENDATION

Results indicate that VSU-modified mY3 is more suitable than COGENT for the *in vitro* culture of embryos of the normal coconut types ALD and BAYT and the hybrid makapuno VMAC1. This was evidenced by significantly higher germination rates and better development of plantlets in the former medium. However, the specific factor(s) contributing to mY3's suitability cannot

be determined due to the limitation in the experimental set-up. Said factors should be determined through further experimentation.

Slight variation in percentages of germination of the three coconut types showed genotypic differences in response to the germination medium but these were not significant. Obviously, since mY3 was tailored for the hybrid makapuno, this medium proved to be the best suited to VMAC1.

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