THE GROWTH OF COCONUT "MAKAPUNO" EMBRYOS *IN VITRO* AS AFFECTED BY MINERAL COMPOSITION AND SUGAR LEVEL OF THE MEDIUM DURING THE LIQUID AND SOLID CULTURES*

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ABSTRACT

Excised coconut *makapuno* embryos were sequentially cultured from liquid to solid medium using modified White's basal medium and Murashige and Skoog's medium with varying levels of dextrose and other supplements. A low salt and high sugar concentration (Wo 8D) in the initial liquid medium and a high salt and moderate sugar concentration in the first transplant medium (Mo 4D) was found to be optimum for a balanced shoot and root development. During the liquid culture not all of the sugar supplied at 8% was used up metabolically; a portion of it was active only as an osmotic agent. In the first transplant medium dextrose seems to be required solely as nutrient source.

INTRODUCTION

With the modified White's basal medium and 2% dextrose in the initial liquid culture and the first solid transplant, a substantial amount of shoot and root growth of coconut *makapuno* embryos was obtained.[1] However, this growth is still far from optimum. The induction of extensive root development without adverse effect on shoot growth was observed when the sugar in the first or second solid transplant was increased to 8%[2].

Sugar requirements of embryos in culture have been reported to vary with the stage of embryo development. The role of sugar in the culture media is not merely nutritional; sugar also acts as an osmotic agent. The osmotic role of sugar as well as nutrients for plant embryos has been considered in the culture of barley embryos[3], Datura[4], Capsella[5,6] and cotton[7]. Mannitol was used by several workers to regulate the osmotic value of the culture media[3,4,5,6]. On the other hand, Ziebur and Brink[8] employed casein hydrolyzate as an osmotic agent.

This study aims to establish the optimum level of sugar and type of basal medium during the initial liquid and first solid transplant. It also attempts to delineate the role of sugar in both stages of culture.

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MATERIALS AND METHODS

Excised coconut *makapuno* embryos were sterilized and inoculated aseptically according to the method of de Guzman[9] and subjected to a sequential culture from liquid to solid medium[1]. Modified White's basal medium[10] (Wo) with varying levels of dextrose was used as the initial liquid medium. For the transfer solid medium, White's medium; Murashige and Skoog's medium (Mo)[11] with varying levels of dextrose was used with or without coconut water (c), indoleacetic acid (IAA), and nitrogen (N) supplements.

In some experiments, the osmotic condition of the medium was controlled with the use of mannitol and casein hydrolyzate in both the liquid and solid media.

Observations on shoot and root growth were made 8 weeks after transfer to the solid medium. Results were analyzed statistically using the randomized complete block design.

RESULTS AND DISCUSSION

Effect of mineral composition of the transplant medium. As shown in table 1, shoot and root growth is significantly less in Wo at any dextrose level.

Table 1. *Response of coconut* makapuno *embryos initially cultured in White's medium with 4% dextrose and transferred to 2 types of basal medium with varying sugar levels.*[a]

Transfer medium	Percent germination[b]	Percent rooting[c]	Ave. root length[d] (mm)	Ave. shoot length[e] (mm)	Ave. no. of leaves[f]
Wo2D	82.20	80.53	5.50	11.70	2.16
Wo4D	73.13	100.00	7.83	10.73	2.43
Wo8D	69.76	64.26	7.20	5.60	1.40
Mo2D	81.70	79.60	4.20	18.40	2.26
Mo4D	70.00	84.73	22.03	31.10	3.06
Mo8D	73.86	87.26	49.83	22.66	2.73

[a]Based on 3 replicates

[b]NS

[c]NS

[d]LSD (0.05) = 14.62
LSD (0.01) = 20.50

[e]LSD (0.05) = 4.80
LSD (0.01) = 6.73

[f]LSD (0.05) = 0.70
LSD (0.01) = 0.99

In Mo, shoot and root growth differences were significant between sugar levels, this was not the case in Wo. Further, there was an inverse relationship in shoot length in Wo as dextrose level was increased from 2% to 8%; this was not the case with Mo. Both shoot and root development was significantly less at Mo 2D than in Mo 4D and Mo 8D. Greatest root growth was attained in Mo 8D, while shoot growth was maximum at Mo 4D.

The above results indicate that coconut *makapuno* embryos require high amounts of mineral nutrients in the transplant medium for optimum shoot and root development. Murashige and Skoog's medium is high in nitrogen and potassium and is about 10 times more concentrated than White's medium. It has been reported to be superior to White's medium in fostering the differentiation of shoot and root[12,13]. In orchid tissue culture, high salts-media (Braun's and Murashige and Skoog's) supported faster initial growth rate[14].

Effect of varying sugar levels in the initial and transplant medium. Growth of embryos sequentially cultured in media with constant, low to high, or high to low sugar levels is shown in table 2. In this experiment, the transfer medium was Murashige and Skoog's basal medium. Percentage germination did not show significant differences between treatments. Lowest rooting percentage was attained when embryos were constantly cultured in 2% dextrose. This was significantly lower than the other treatments. With Mo 2D as trans-

Table 2. *Growth of coconut* makapuno *embryos initially cultured in White's medium and transferred to Murashige and Skoog's medium with varying levels of dextrose in both media.*[a]

Liquid medium	Solid medium	Percent germination[b]	Percent rooting[c]	Ave. root length[d] (mm)	Ave. shoot length[e] (mm)	Ave. no. of leaves[e] (mm)
Wo2D	Mo2D	73.17	39.85	4.30	13.85	1.67
	Mo4D	80.22	79.57	11.47	29.17	2.95
	Mo8D	89.70	87.82	40.50	20.27	2.05
Wo4D	Mo2D	87.75	65.60	5.70	13.80	1.60
	Mo4D	78.55	87.50	10.97	19.37	2.32
	Mo8D	77.35	69.37	44.87	16.85	1.85
Wo8D	Mo2D	83.32	73.35	7.60	21.05	2.02
	Mo4D	89.37	73.97	23.57	31.67	2.57
	Mo8D	68.25	87.20	59.07	19.55	2.10

[a]Based in 4 replicates

[b]NS

[c]LSD (0.05) = 26.36

[d]LSD (0.05) = 16.90
LSD (0.01) = 22.90

[e]LSD (0.05) = 9.32
LSD (0.01) = 12.63

[f]LSD (0.05) = 0.73
LSD (0.01) = 0.98

plant medium, preculture in Wo with 4% and 8% dextrose greatly improved percent rooting.

Transfer of embryos initially cultured in varying sugar levels to Mo 2D resulted in very poor root growth which did not differ significantly from each other. High sugar during the liquid culture did not alleviate the deficiency with Mo 2D. When the transfer medium was Mo 8D, root growth was significantly greater than those transferred to lower sugar, regardless of sugar level in the initial liquid medium. However, taken altogether, root growth was least when the initial liquid medium was Mo 2D and greatest with Mo 8D.

At any sugar level in the initial liquid medium, shoot growth was greater when transplanted to Mo 4D than to Mo 2D. However, Mo 8D did not favor optimum shoot growth as compared to those transferred to Mo 4D. Leaf number was least when transplanted to Mo 2D and greatest when the transfer medium was Mo 4D.

Results of this experiment show that regardless of the sugar level in the initial liquid medium, 2% dextrose in the transplant medium is insufficient for satisfactory shoot and root growth. Shoot growth was optimum at 4% dextrose and root growth at 8% dextrose in the transplant medium. Initial culture in liquid Wo 8D and subsequent transfer to Mo 4D gave the most balanced shoot and root ratio. Predominant factor controlling root growth is the sugar level during transplant; however, for any given sugar level at transplant, increasing sugar during the initial culture may give a favorable effect.

Effect of supplements to the transplant medium. Table 3 shows the effect of the transfer of embryos to Murashige and Skoog's basal medium with different levels of dextrose with (Mc) or without coconut water (Mo), IAA, and 500 ppm nitrogen as supplements. Results of this experiment confirm the previous findings that increased root growth is attained with increasing sugar levels during the transplant stage. Optimum shoot growth was attained by transfer to Mo 4D. Addition of coconut water to Mo 8D resulted in a decrease of both shoot and root growth, which was further decreased with the addition of IAA. Further addition of 500 ppm nitrogen partially counteracted the inhibitory effect of IAA. However, germination percentage in this medium was lowest among the treatments. There was not much difference in rooting response between treatments. This is contradictory to the observations made by Balaga and de Guzman[1] that Murashige and Skoog's medium negates the inhibitory effect of coconut water and IAA.

Effect of addition of mannitol and casein hydrolyzate in the initial and transplant media. From the preceding experiments, it was shown that 8% dextrose in the initial liquid medium and 4% dextrose in the transplant

in the inhibition of shoot and root growth. Germination was drastically inhibited in 2% dextrose and mannitol compared to that with 2% dextrose only. Germination was also considerably inhibited in the high sugar medium. This indicates the inhibitory effect on germination of high osmotic condition whether this be due to sugar or a combination of sugar and mannitol.

Table 4. *Growth of coconut* makapuno *embryos initially cultured in White's liquid medium with varying levels of dextrose and mannitol and then transferred to Mc + 10 IAA with 4% dextrose.*[a]

Sugar level		Percent Germination	Percent rooting	Ave. shoot length[b] (mm)	Ave. root length[c] (mm)
Dextrose	Mannitol				
2%	-	64.02	89.62	10.40	9.73
4%	-	79.62	94.44	13.50	13.50
8%	-	80.05	97.22	14.26	11.60
2%	6.5%	76.07	92.80	21.30	11.76
4%	4.4%	70.55	97.43	15.93	20.50

[a]Based on 3 replicates

[b]LSD (0.05) = 12.98
LSD (0.01) = 10.24

The role of sugar in the initial and transplant media. The role of sugar in the growth of *makapuno* embryos can be evaluated separately in the two phases of culture. The trend is that increasing the osmotic condition of the liquid medium containing 2% and 4% dextrose with mannitol can improve subsequent embryo growth (table 4). The fact that root growth with 4% dextrose and mannitol is better than 8% dextrose only during the liquid culture, indicates that not all of the sugar supplied at 8% was used up metabolically; a portion of it was active only as an osmotic agent. On the other hand, addition of mannitol to increase the osmotic condition of the transfer media with 2% and 4% dextrose resulted in an inhibition of both shoot and root growth (table 5). At this stage, dextrose seems to be required solely as a nutrient source.

This implies that a high osmotic value is favorable for coconut *makapuno* embryos during the initial liquid culture. Balaga and de Guzman[1] have reported that at this stage, the embryo undergoes further embryonic growth. Many workers have stressed the role of a high osmotic value for continued embryonic growth of immature plant embryos in culture. In *Datura*, different embryo stages require different osmotic values in the medium, and decreases as the embryo stage advances[4]. Similar requirements have also been noted in

medium gave the most balanced shoot and root growth. To test the role of sugar in the growth of makapuno embryos, mannitol and casein hydrolyzate were used to modify the osmotic condition of the culture medium used. Mannitol was added to the medium with 2% and 4% dextrose to equal the osmotic value of the medium with 8% dextrose. This was done for both the initial liquid and the transfer medium. Casein hydrolyzate was also added to the initial and transfer media in like manner.

Table 3. *Response of coconut* makapuno *embryos initially cultured in White's liquid medium with 4% dextrose and transferred to the treatments indicated below*[a].

Transfer medium Type	Sugar medium	Percent germination	Percent rooting	Ave. shoot length[b] (mm)	Ave. root length[c] (mm)
Mo	2%	90.97	8.94	16.80	5.55
Mo	4%	86.92	100.00	20.85	24.45
Mo	8%	80.73	96.96	11.35	41.52
Mc	8%	71.81	88.57	9.68	22.80
Mc + 10 IAA	8%	74.23	89.44	6.81	13.32
Mc + 10 IAA + 500 N	8%	63.16	84.52	10.78	26.05

[a]Based on 3 replicates

[b]LSD (0.05) = 7.78
LSD (0.01) = 11.07

[c]LSD (0.05) = 17.99
LSD (0.01) = 25.60

Table 4 shows the growth of coconut *makapuno* embryos initially cultured in White's liquid medium with varying levels of dextrose and mannitol. Incorporation of mannitol in the medium with 2% and 4% dextrose resulted in an increase in both shoot and root growth over the control. Root was longest in the medium with 4% dextrose plus mannitol. There were no distinct differences in germination percentage between treatments.

When mannitol was added to the transfer medium with 2% dextrose (table 5), there was a marked reduction in shoot and root growth compared to both the 2% and 8% dextrose treatments. Even with 4% dextrose plus mannitol, root growth was significantly less than that in 8% dextrose. Similar experiments using casein hydrolyzate in place of mannitol when applied to the liquid and solid medium to regulate the osmotic condition, likewise, resulted

Capsella[5], barley[3] and cotton embryos[7].

Table 5. *Response of coconut* makapuno *embryos initially cultured in White's mediun with 4% dextrose to varying dextrose and mannitol concentrations in the transfer medium.*[a]

Sugar level		Percent germination	Percent rooting	Ave. shoot length[b] (mm)	Ave. root length[c] (mm)
Dextrose	Mannitol				
2%		82.20	92.59	9.50	3.30
2%	6.3%	30.36	77.77	1.45	1.53
4%	4.2%	58.42	98.79	3.09	4.20
8%		47.35	92.59	3.23	8.56

[a]Based on 3 replicates
Transfer medium = Mc + 10 IAA

[b]LSD (0.05) = 4.00
LSD (0.01) = 6.07

[c]LSD (0.05) = 3.03
LSD (0.01) = 4.60

This study shows that for the coconut *makapuno* embryo a low salt and high sugar concentration (Wo 8D) in the initial liquid medium and a high salt and moderate sugar concentration (Mo 4D) in the first transplant medium is optimum for a balanced shoot and root development.

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