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STUDIES ON THE RELATIONSHIP BETWEEN PROTOCORM DENSITY AND ITS MULTIPLICATION RATE OF *ARANDA* CHRISTINE 80 CULTURED *IN VITRO*

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RINGKASAN

'Protocorm' dari jenis *Aranda* Christine 80 telah disuntik ke dalam media cair Modified Vacin and Went. Empat perlakuan yang mengandungi 5, 10, 20 dan 40 protocorm se kelalang, telah dikulturakan di dalam media tersebut selama tiga, enam dan sembilan minggu. Data telah dianalisa dengan menggunakan kaedah perbandingan bagi perbezaan kelok-kelok pertumbesaran. Adalah dijangkakan bahawa takat cantuman optima yang mungkin diperolehi untuk mencapai berat pertumbesaran yang maksima terjadi dengan 10 bilangan protocorm se kalalang pada minggu ketiga.

INTRODUCTION

Since MOREL (1960) published his first report on the successful propagation of *Cymbidium* orchid through tissue culture method, this technique has been well developed and adopted in commercial production of many desirable orchid genera and hybrids (MURASHIGE, 1974; RAU, 1977). Research has been carried out by many researchers throughout the world which contributes to the rapid improvement on the cultural technique. However, a question is often raised among orchid growers regarding the optimal number of orchid protocorms to be inoculated into the culture medium and its respective cultural period in which a maximum growth rate or protocorms can be achieved. This study was therefore conducted to provide the answer, by using one of our common types of orchid, *Aranda* Christine 80 as experimental material. The results obtained will serve as a useful guideline in promoting efficiency on mass clonal propagation or orchid through tissue culture technique.

MATERIAL AND METHODS

Protocorms of *Aranda* Christine 80 being cultured *in vitro* for one and a half years, was further subcultured twice before being selected as experimental materials. This ensures that the protocorms used in the experiment maintained uniformity in their physiological and morphological conditions.

Four treatments with three replicates were inoculated into the culture medium. The treatments were 5, 10, 20 and 40 protocorms respectively with an average weight of 0.15 gram per protocorm. Weighing was performed under aseptic conditions using an electric digital sauter top pan balance. Fresh weight increases were recorded on the third, sixth and ninth week after initial culture. Culture medium was unchanged from initial inoculation throughout the experiment for all treatments.

A modified liquid Vacin and Went medium (1949) which was found to be excellent for protocorm multiplication in *Aranthera* orchid (CHUA *et al,* 1980) was used. The pH was adjusted to 5.2 $\pm$ 0.2 prior to autoclaving at 1.5 kg/cm^2 for ten minutes. Protocorms were cultured in 250 ml. flasks each filled with approximately 60 ml. liquid medium. They were constantly agitated on a Lab-line orbital shaker at 120 rpm and maintained at 27 $\pm$ 2°C under 2000 lux illumination from white flourescent lamps at 16 hours light and eight hours dark cycle.

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TABLE 1. THE OBSERVED WEIGHT (GRAM) OF VARIOUS PROTOCORM NUMBER TREATMENTS VS CULTURAL PERIOD (WEEK)

Weeks Observed	Replicate Number	Number of Protocorms Inoculated per Flask			
		5	10	20	30
0 Week (initial weight)	1	0.10	0.18	0.30	0.60
	2	0.10	0.18	0.30	0.58
	3	0.09	0.18	0.30	0.60
3rd Week	1	0.43	2.44	2.33	3.80
	2	0.76	1.46	2.58	4.61
	3	0.84	1.91	2.80	3.83
6th Week	1	4.65	6.42	6.23	6.16
	2	4.06	5.53	6.86	8.82
	3	4.13	5.21	6.63	6.61
9th Week	1	6.25	7.18	6.62	6.00
	2	5.67	6.57	7.71	9.29
	3	5.65	5.99	6.73	7.41

RESULTS AND DISCUSSION

The weight increase of protocorms in each treatment was hypothetically generated beginning from three weeks until 36 weeks based on the observed data recorded on the 3rd, 6th and 9th week (*Table 1*). Results obtained were used for the comparison of protocorm growth rate among treatments.

Incremental multiplicative factor method was used to generate the data until 36 weeks. This factor is the ratio of the average weight for the week recorded to the initial average weight. For the three weeks culturing period, the weeks are taken to be multiples of three beginning on the third week. For the six weeks culturing period multiples of six beginning on the sixth week, and for the nine weeks culturing period multiples of nine beginning on the ninth week.

The hypothetical weight values of the successive weeks are the results of the multiplication between the incremental factors and of values of the weight in the previous week. The results of analysis is stated in *Table 2.*

From *Table 2,* it is clearly shown that among various protocorm treatments (number of protocorms per flask), the three weeks culturing period has obtained the highest hypothetical weight increase as compared to the six weeks and nine weeks cultures. Hence, it can be determined that three weeks culture is an optimal duration to achieve a faster protocorm multiplication rate.

Based on this recommended three weeks culturing period, another hypothetical weight is generated to compare the growth rate among various protocorm number treatments. The values generated for the larger protocorm number per flask are divided throughout by the incremental factor. The incremental factor is the ratio of the average weights of the smaller protocorm number per flask to that of the larger number of protocorm. The result of the hypothetical weight

TABLE 2. THE HYPOTHETICAL WEIGHT (GRAM) GENERATED ON THE 3-WEEKS, 6-WEEKS AND 9-WEEKS CULTURES IN VARIOUS PROTOCORM NUMBER TREATMENTS

Treatments (No. of	Weeks (Hypothetical)	Culturing Period		
		3 Weeks	6 Weeks	9 Weeks
5	18	1.1×10^4	8.4×10^3	3.5×10^2
	36	1.3×10^9	7.3×10^8	1.3×10^6
10	18	2.8×10^5	5.8×10^3	2.4×10^2
	36	4.3×10^{11}	1.9×10^8	3.2×10^5
20	18	1.2×10^5	3.2×10^3	1.6×10^2
	36	4.7×10^{10}	3.3 4 10^7	9.0×10^4
40	18	6.5×10^4	1.1×10^3	9.7×10^1
	36	7.1×10^4	1.9×10^6	1.6×10^4

TABLE 3. THE HYPOTHETICAL WEIGHT (GRAM) GENERATED ON 18 WEEKS AMONG VARIOUS PROTOCORM-NUMBER TREATMENTS FOR THE 3 WEEKS CULTURING CYCLE

Treatments	Weight Generated
P5 (5 protocorms per flask)	1.1×10^4
P10 (10 protocorms per flask)	1.5×10^5
P20 (20 protocorms per flask)	3.8×10^4
P40 (40 protocorms per flask)	1.0×10^4

generated on the 18th week for all treatments are presented in *Table 3* indicating that the treatment with 10 protocorms per flask (P10) has generated the most weight increase among all treatments.

From the two hypothetical results derived from the observed weight record (*Table 2* and *3*), it can thus be concluded that by inoculation of 10 protocorms (average weight 0.15 gram/protocorm) per culture flask a maximum protocorm multiplication rate can be attained in a three weeks culturing cycle. Hypothetical growth curves shown in *Figure 1* has further illustrated the superiority in the multiplication rate of a '10 protocorms — three weeks culture' combination as compared to the others.

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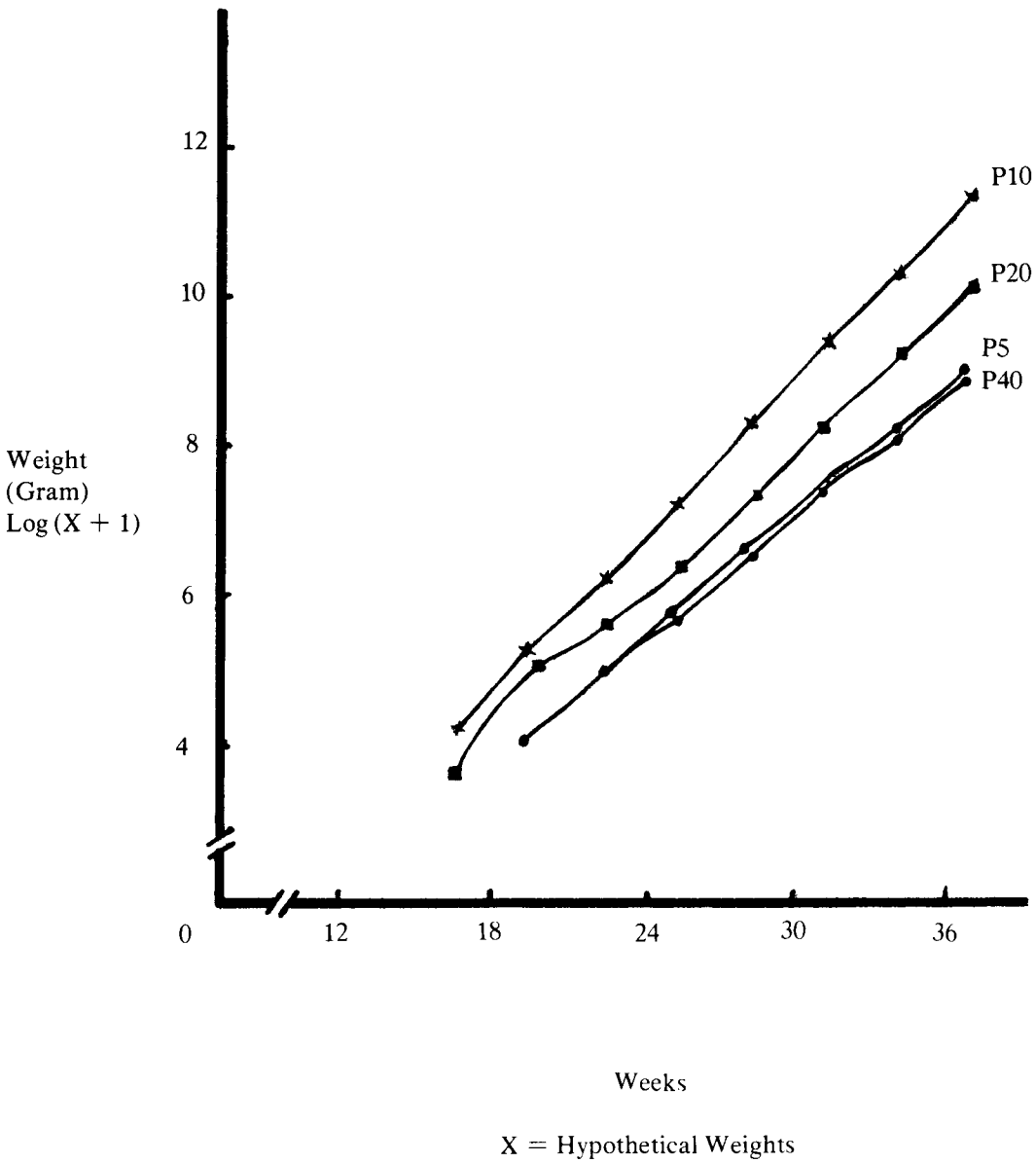

Figure 1: Hypothetical growth curves of protocorm — number treatments in 3-weeks culturing cycle

SUMMARY

Protocorms of *Aranda* Christine 80 were cultured on Modified Vacin and Went liquid medium. Four treatments with 5, 10, 20 and 40 protocorms per flask were cultured for a duration of 3, 6 and 9 weeks respectively. Results indicate that treatment with 10 protocorms per flask cultured for 3 weeks has attained the highest multiplication rate as compared to the others.

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