琉球大学学術リポジトリ

カトレイトニア組織の低温保蔵とその長期化に及ぼす蔗糖およびココヤシ液の影響(農学科)

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Effects of Sucrose and C W on the Survival of P L B Tissues of *Cattleytonia* under Cold Storage

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Summary

This experiment was carried out in order to clarify the possibility of using of P L B tissues of orchid as germplasm under cold condition for long time. P L B tissues of *Cattleytonia* Rosy Jewel obtained from apical meristem were used as materials. After storage on 4-5°C temperature with some combinations of culture, P L B tissues transfered to normal culture condition on 25-27°C temperature. The results of the experiment concerning the relationship between culture conditions and survival tatio are as follows.

1. P L B tissues of *Cattleytonia* could survive for 4 weeks on 4-5°C temperature though this plant does not have tolerance for cold temperature because it originated from a tropical predecessor plant.

2. Immersed condition in a liquid medium was much better for the survival of P L B tissues than on a surface of agar medium.

3. P L B tissues in the liquid V W medium to which 150ml/1 of C W, 20g/1 of sucrose had been added could survive for 7 weeks at 4-5°C temperature.

4. Survival duration at 4-5°C temperature was extended to 15 weeks in a liquid medium to which 100g/1 of sucrose had been added.

This paper is a part of my research which I carried out in 1982 at the University of Hawaii during my time as a visiting researcher sponsored by the Ministry of Education. This research was also reported at the Conference of the Japanese Society for Horticultural Science in Kanagawa, 1984 and at the 11th World Orchid Conference in Miami, 1984.

I Introduction

Works with carrot (4), strawberry (5), chrysanthemum (2) and sugar cane (3) indicate that the storage of germplasm in low temperature may provide experimental and practical advantages. If this technique can be applied to orchids, it might be hightly beneficial for preservatjon of valuable

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species or hybrids. In orchids the technique of meristem culture is highly advanced. However, protocorm like bodies (P L B) of orchids is not easy to keep frozen or at low temperatures because most valuable orchids come from the tropics.

This experiment was done to show how P L B tissues of *Cattleya* alliance respond to cold temperature and how sucrose can modify survival.

II Materials and Methods

P L B of *Cattleytonia,* Rosy Jewel (C. bowringiana X *Broughtonia sanguinea*) propagated from apical meristem were used. All P L B masses used as materials were prepared by subculture for 3 -4 weeks after cutting into small pieces. This was necessary for obtaining uniform materials both morphologically and in size. The medium used for subculture was modified Vacin and Went (V W) with 150ml/1 of coconut water (C W).

Two experiments were planned at about the same time. One of the experiment was carried out to get a more suitable culture for cold storage. On this point, four groups of culture methods were compared : solid and liquid with and without C W. All vials which incubated twelve pieces of P L B for liquid medium and a piece of P L B for agar medium using by small test tubes were maintained 4-5°C temperature in the dark. After incubation, twenty four pieces from two flasks for liquid and twenty pieces from the same number of test tubes for solid of each group were transflasked weekly to solid medium which was the same medium with C W. Cultures were maintained under continuous illumination at room temperature (25-27°C). After 6 weeks, cultures were removed from vials and checked for survival grade of activity and sprouting, fresh and dry weight.

Another experiment was carried out according to get data about the relationship between concentration of sucrose and survival of P L B pieces under cold storage. Experiments were prepared at the following cocentrations 0, 20, 40, 70, 100g/1 limited to liquid medium with C W. Basic medium and methods including the number of pieces on each group were the same as described above. In addition, fresh and dry weight of one group (24 pieces) from selected P L B pieces used as material were 149.8 ± 20.7 mg and 15.3 ± 2.5 mg (confidence limits at 95% level) respectively.

III Result

1. Comparison between solid and liquid, with and without C W

Results obtained from the experiment related culture method are shown in Fig. 1 and Table 1. Survival ratio in liquid medium was higher than on solid. In comparison no tissues survived after 5 weeks storage on solid medium. During the first 3 weeks of storage at 4-5°C temperature there was no difference between solid and liquid, however, the survival ratio was reduced rapidly in the following 2 weeks.

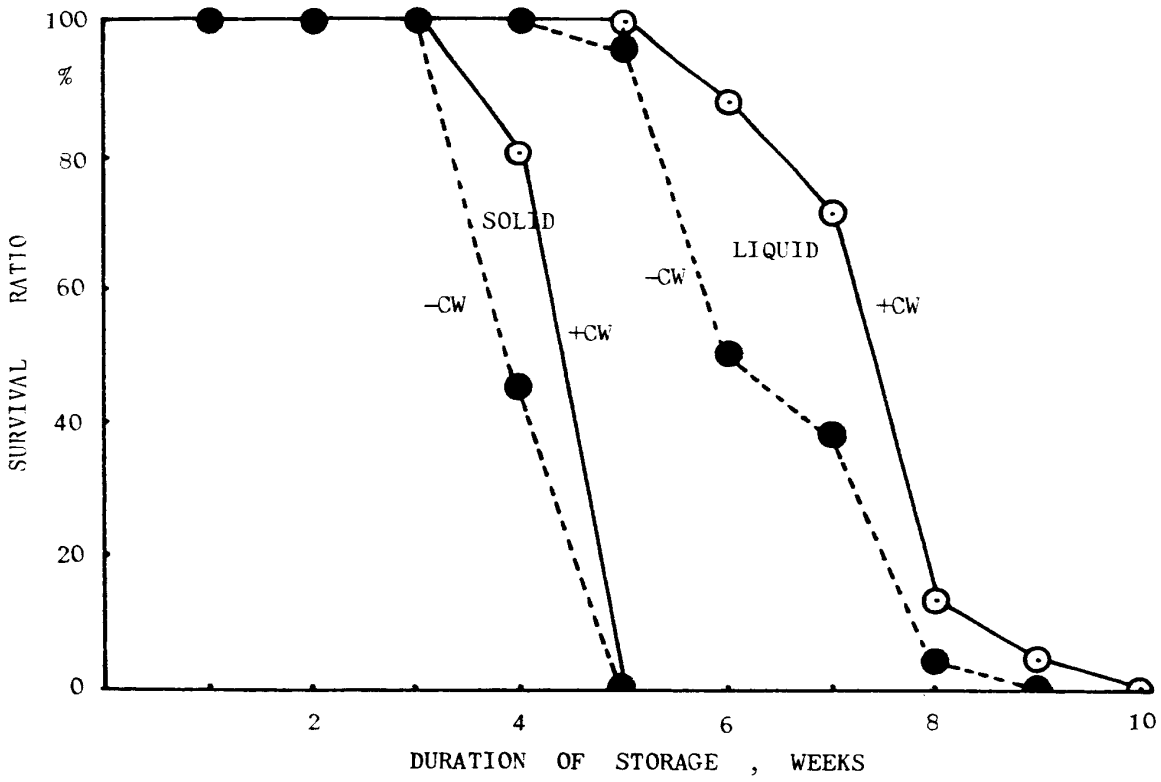

Fig. 1. Comparison between solid and liquid, with and without coconut water in the medium on survival of P L B tissues of *Cattleytonia* under cold storage on 4-5°C

Table 1. Comparison between solid and liquid, with and without coconut water in the medium on the growth of P L B pieces of *Cattleytonia* during 6 weeks culture after the cold storage on 4-5°C. Each value shows mean dry weight which obtained 72 hours during on 70°C.

Duration of Storage	With C W		Without C W	
	Solid	Liquid	Solid	Liquid
weeks	mg	mg	mg	mg
1	60.4	-	60.8	-
2	59.5	72.8	47.3	81.4
3	46.6	-	52.0	-
4	35.0	45.9	42.0	33.0
5	0	31.5	0	20.4
6	0	25.4	0	22.3
7	0	20.3	0	14.7
8	0	15.7	0	16.0
9	0	17.0	0	0
10	0	0	0	0

Effect of coconut water was not very different with liquid or solid as shown in Fig. 1. Survival was higher in medium with C W in both liquid and solid. Dry weight obtained from same experiment are shown in Table 1. This characteristic was measured as a standard of activity of tissues. The tendency generally was the same as explained above for survival ratio. Fresh weight (that table is ommitted here) shows the same tendency as dry weight, just difference is observed on about ten times of values.

2. Effects of various concentration of sucrose on the survival under the cold storage

Results of survival ratio obtained from the experiment related with sucrose under 4-5°C temperature are shown in Fig. 2. On the whole, the effects of sucrose for cold storage were significant. Some P L B pieces with 100g/l sucrose survived 15 weeks storage. This phenomenon is noteworthy because *Cattleytonia* is not only a tropical plant but the materials used here were P L B. This is much different with seed which is in dormant stage. P L B pieces can survive for 7 weeks in the medium without supplemental sucrose.

The period of survival in cold storage generally increased with concentration of sucrose. This tendency is very clear in Fig. 2. For instance, the time of survival was reduced less than 50% in 5, 7, 8, 9, 11 weeks respectively at each concentration of 0, 20, 40, 70, 100g/l sucrose.

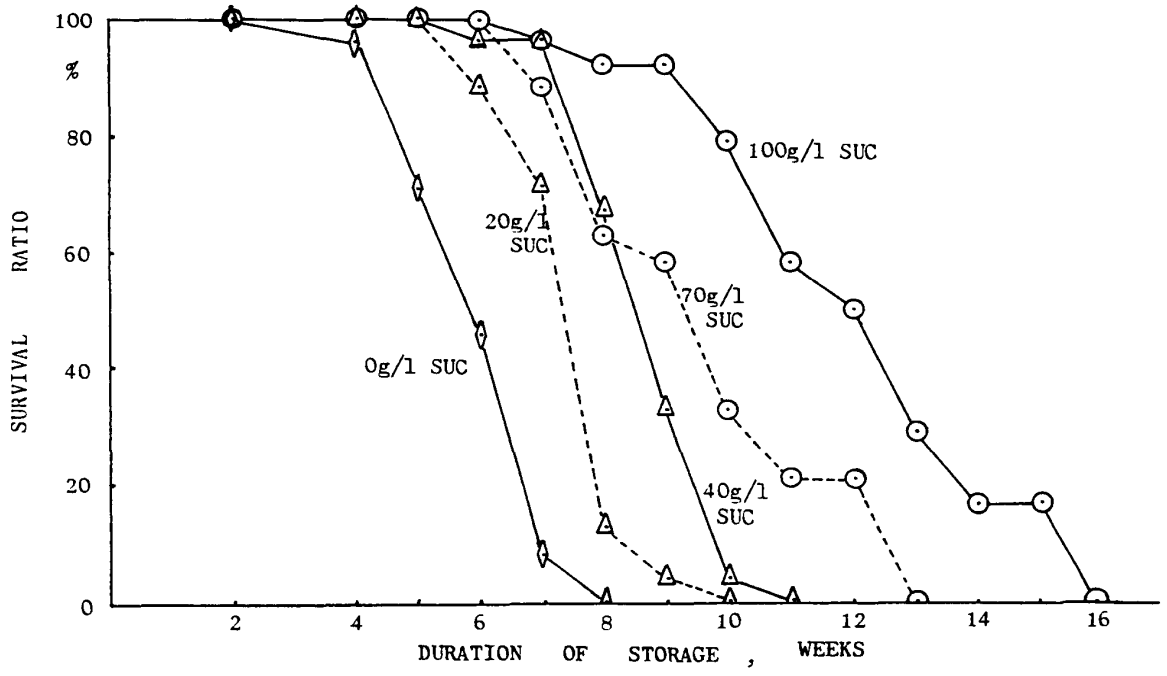

Fig. 2. Effects of various concentration of sucrose on survival of P L B tissues of *Catteleytonia* under cold storage on 4-5°C.

Table 2. Effects of various concentration of sucrose on the growth of P L B pieces of *Cattleytonia* during 6 weeks culture after the cold storage on 4-5°C.

Duration of Storage	Mean Dry Weight, mg					
	Concentration of Sucrose, g/l					
weeks	0	20	40	70	100	LSD. 05
2	70.5	72.8	80.2	88.6	86.9	24.1
4	22.9	45.9	60.8	62.0	73.5	19.2
5	21.4	31.5	31.1	46.5	52.1	12.2
6	21.4	25.4	29.8	44.0	54.8	14.1
7	15.0	20.3	23.6	26.5	31.9	8.8
8	0	15.7	20.1	22.5	26.0	9.1
9	0	17.0	16.8	18.5	24.3	7.4
10	0	0	14.0	16.8	20.5	5.3
11	0	0	0	16.8	20.1	5.5
12	0	0	0	14.0	18.5	7.4
13	0	0	0	0	19.6	
14	0	0	0	0	20.8	
15	0	0	0	0	18.8	
16	0	0	0	0	0	

Dry weight obtained from same experiment is shown in Table 2. The tendency generally was the same as explained above for survival ratio. Dry weight of cultures containing high concentrations of sucrose showed higher value in any time period when compared with those of low concentrations of sucrose. Such a result was obvious at the previous term of 6 weeks storage. After that, the P L B pieces obtained through cold storage showed similar size briefly. Moreover these were the same shape morphologically. As for the small sized P L B obtained at short term storage, they showed more advanced stage toward seedlings.

IV Discussion

There is no easy method to maintain tissues obtained from tropical plants in cold storage. If preservation of plant tissue as germplasm becomes feasible under cold storage, there are many advantages not only in experimentation but also on the practical side. Organs, tissues and cells of plants cannot withstand variation of circumstances because these are active. This is very different from seeds or pollen which are in dormant state. However, there are several reports about the preservation of the tissue or cell in various kind of the plants (4, 5, 2) including sugar cane (3) which is a tropical plant.

This experiment was carried out to test the responses to cold temperature and the relationship between the concentration of sucrose and survival ratio with P L B tissues of *Cattleytonia.* Survival under frozen condition was checked first separately from main experiment. The result showed completely negative effect. However, at 4-5°C which is the temperature of a common refrigerator, P L B pieces could survive several weeks without growth.

Survival was very different with each culture method. Liquid medium is much better than solid. It might be suggested that the tissue could absorb sugar and minerals more easily from the surrounding medium. Consequently, the activity of tissue kept at high level of osmotic pressure in tissue increased much. It may be the same as the result obtained from main experiment which showed high survival ratio as concentration of sucrose in medium increased (Fig.2, Table 2). The P L B tissues in the medium containing 100g/l sucrose could survive for 15 weeks at 4-5 °C temperature. That was about double compared with medium without sucrose (but it contained less sucrose from coconut water). P L B tissues after 15 weeks storage showed sprouting of shoots even though survival was low and only a small part of the tissue was alive. It may be that in this kind of cold storage tissue survival is more important because it can easily propagate upon return to normal culture conditions.

Effectiveness of C W added medium for cold storage might be due to sugars and various kind of plant hormones contained in this water (6). DMSO was one of the cryoprotectants used for same materials at several concentrations under the same method with 20g/l sucrose. But is showed no positive effects. Probably it needs combined treatment with high concentration of sucrose.

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カトレイトニア組織の低温保蔵とその長期化に及ぼす蔗糖およびココヤシ液の影響

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要　　約

ランのPLB組織を，ジャームプラズムのひとつとして長期保蔵に利用できないかと考え，同組織の低温下における保蔵法を検討した。実験材料には，茎頂組織培養法によって増殖したカトレイトニアRosy JewelのPLB組織を用いた。4～5℃の冷蔵庫内にいくつかの培養条件を組合わせてＰＬＢ組織を保蔵し，その後通常の培養条件下に戻して生存率を検討した。得られた結果は次のとおりである。

1．カトレイトニアは耐寒性をもたない熱帯性植物であるにもかかわらず，そのPLB組織は，4～5℃の低温下で4週間の保蔵に耐えられることが示された。

2．PLB組織はカンテン上に置床されることより，液体培地に沈められる条件が適しており，それぞれCW添加により生存期間が若干延長されることが認められた。

3．Vacin-Went培地にCW150ml/l，蔗糖20g/lの添加した培地において，50%以上の生存率が維持される限度は7週間であった。

4．上述の生存期間は，蔗糖100g/lが添加されることにより15週まで延長されることが確認された。

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