

## Growth Reduction of Papaya Plantlets for *In Vitro* Germplasm Preservation

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### ABSTRACT

Papaya plantlets, line Pb1-R1, were cultured on modified on Murashige and Skoog media supplemented with different concentration of chloro choline chloride (CCC) namely 0, 50, 100, 250, 500, 1000, 1250, 1500, 1750 and 2000 mg/l and cultured under 4 levels of temperature namely 4°C, 10°C, 15°C and 27°C. Under room temperature, papaya plantlets cultured on media supplemented with each level of CCC could grow for 3 months. On the other hand, under low temperature, 4°C 10°C and 15°C, papaya plantlets could grow for 6 months. Culture papaya plantlets under 15°C without CCC is the suggested condition for preservation of papaya germplasm for 6 months.

**Keywords:** papaya plantlet, germplasm preservation

### INTRODUCTION

Papaya usually propagates by seeds. Under this method of propagation, papaya can not retain selected characteristics because papaya is a dioecious plant. Therefore, seed of papaya is not suitable to be stored for germplasm preservation *in vitro* preservation of plantlets is the alternative method for this problem.

Litz and Conover (1978) could induce plantlets from shoot tip and lateral buds. Burikam *et al.* (1987) repeated the experiment on two papaya varieties; Khag Dum and Solo. Various methods were used for germplasm preservation. Sugarcane plantlet was preserved under low temperature in sugarcane (Lersrutaiyotin *et al.*, in press), by using growth suppression chemicals (Sonthichai *et al.*, 1992) and by culture on limiting concentration of inorganic nutrient and growth promotor supplement (Lersrutaiyotin *et al.*, in press). In this study, plantlets of papaya were preserved by using chloro choline chloride under low temperature.

### MATERIALS AND METHODS

**1. Induction of plantlets from papaya shoot tips**  
shoot tips and lateral buds of papaya line Pb1-R1 (lincensed by Siam Agroindustry, Pineapple, Ltd.) were surfaced sterized by 10% Clorox with 0.1% Tween 20 for 20-30 minutes. After rinse in sterile distilled water for three times, the explants were put onto MS media supplemented with 1 mg/l kinetin or 1 mg/l kinetin and 0.1 mg/l NAA with 7 mg/l agar at pH 5.8 (Murashige and Skoog, 1962). The cultures were incubated in a growth room at 27°C under 16 hours photoperiod.

**2. Culture papaya plantlets on media supplemented with CCC**

After culture on plantlet induction media for 2 months, papaya plantlets were subcultured onto multiplication media, modified Murashige and Skoog media supplemented with 80 mg/l adenine sulfate, 0.5 mg/l BA, 0.1 mg/l NAA ( naphthalene acetic acid), 30g/l sucrose and 0.8% by weight of agar at pH 5.8.

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After subcultured on multiplication media for 2 weeks, papaya plantlets were subcultured on the same media containing 0,50,100,250,500,1000,1250,1500,1750, or 2000 mg/l. concentration of CCC. The culture condition was 2000 lux light intensity, 16 hours photoperiod at 27°C.

### 3. Culture papaya plantlets under low temperature

Papaya plantlets line Pb1-R1 cultured on the multiplication media were subcultured on the same media and cultured under 4 different levels of temperature namely, 4°C, 10°C, 15°C and 27°C. Culture condition in this study was also the same as the former experiment.

Weight of plantlets of both CCC and low temperature experiments were recorded monthly from the beginning of the experiment. The increasing weight was calculated from the difference between the weight of plantlets at first month and the weight of plantlets at the end of each month. Plantlet morphology were also observed.

## RESULTS AND DISCUSSION

### 1. Growth of papaya plantlets on media supplemented with chloro choline chloride

Increasing weight of papaya plantlets cultured

on media supplemented with CCC were shown on Table 1. The increasing weight was about the same at each month in all concentrations of CCC. Therefore, growth of papaya plantlets are not observed after culture *in vitro* for 1 month even culture on media without CCC. For multiplication purpose, papaya plantlets have to be subcultured every 2 weeks.

Different levels of concentration of CCC have slight effect on growth of papaya plantlets. The high concentration of CCC from 100 mg/l up to 2000 mg/l seemed to have the same level of effect on growth reduction while 50 mg/l of CCC showed the less effect on growth reduction of papaya plantlets. However, all of papaya plantlets cultured on media without or with any concentration of CCC could not grow longer than 3 months. After 3 months, all plantlets had yellow stem and the leaves fell down. Contamination of fungi in the plantlets were observed.

### 3. Growth of papaya plantlets under low temperature

Table 2 shows the increasing weight of papaya plantlets cultured under 4 different levels of temperature. The average increasing weight at 4°C was 0.29 while the increasing weight at 10°C was 0.89 and at 15°C was 1.28. This indicated would that the lower the temperature, the lower the level of growth of papaya plantlets be observed. The increasing weight

**Table 1** Increasing weight (g) of papaya plantlets cultured on media with different concentrations of chloro choline chloride (CCC) at each month after starting culture.

CCC concentration (mg/l)	Months <sup>1</sup>			Average
	1	2	3	
0	4.21	3.47	4.66	4.11
50	3.49	3.73	3.35	3.52
100	2.70	1.04	2.16	1.97
250	2.02	2.27	2.68	2.32
500	1.97	1.29	1.21	1.49
1000	3.16	0.83	3.73	2.57
1250	2.07	1.42	1.36	1.62
1500	2.06	1.58	1.95	1.86
1750	3.04	2.23	3.07	2.78
2000	2.46	2.89	1.45	2.27
Average	2.72	2.08	2.56	

<sup>1</sup> mornths after starting the experiment

**Table 2** Increasing weight of papaya plantlets cultured under different level of temperature (°C) at each month after starting culture.

Month	Temperature <sup>1</sup>			
	26	15	10	4
1	4.21	0.69	0.70	0.38
2	3.47	1.07	0.79	0.42
3	4.66	1.76	0.78	0.07
4	-	1.22	0.67	0.18
5	-	1.77	0.93	0.23
6	-	1.17	1.45	0.45
Average	4.11	1.28	0.89	0.29

<sup>1</sup> months after starting the experiment

of papaya plantlets after 1 month at different period of storage was not clearly found for each level of temperature. The ability of growing under *in vitro* condition of papaya plantlets may be observed only within 1 month or less in which temperature level of culture condition is one of the factors that control the growing rate of papaya plantlet. Under low temperature, 15°C, 10°C and 4°C, papaya plantlets could grow for 6 months, after that stem became succulent, the color of leaf turned yellow and the plantlets died without contamination. This indicates that papaya plantlets can survive for 6 months under low temperature with different growing rate at each level of temperature.

### CONCLUSION

Papaya plantlets can be stored *in vitro* for 3 months under room temperature without adding CCC in media. For better vigor and faster recovery, papaya plantlets can be kept at 15°C for a maximum storage life of 6 months under proper reculture in the suitable media.

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