

Effects of CO₂ Enrichment and Sucrose Concentrations on Growth of Papaya Shoots Cultured in Vitro.

Siriwan Burikam and Chada Chaiyasirisuwan¹

ABSTRACT

Study on growth of papaya shoot variety 'Ko Ko' in culture supplemented with different CO₂ concentrations enriched to CO₂ incubator : atmosphere (0.03), 1, 3 and 5%, various concentrations of sucrose added to modified MS medium : 0, 1, 2 and 3%. Two types of bottle covered were used : plastic caps and aluminum foil with membrane filter for gas exchange. The culture were kept in CO₂ incubator under 1,200 lux light intensity, 12 hrs. photoperiod for three weeks. Determination of papaya shoot growth was achieved by calculating percent increment by weight. Papaya shoots cultured in the medium with 1% sucrose and 5% CO₂ enrichment yielded the highest increasing percentage. The result shows that there was no significant difference in weight among sucrose and CO₂ concentrations but there was significant difference among types of the bottle covered.

INTRODUCTION

Higher plants containing chlorophyll in leaves, normally grow by obtaining CO₂ from the atmosphere as a carbon source for photosynthesis, which have been called autotrophic plants. Compare to the plantlets cultured in vitro which have no or little photosynthetic ability (Earle, E. and Langhans, R., 1975; Grout, B.W.W. and Aston, M.J., 1978). Therefore sugars (mostly sucrose, rarely glucose or fructose) are added in the media as a carbon source for plant growth. These are called heterotrophic or mixotrophic plants. So far an in vitro condition always cause some effects to the plantlets such as:- low photosynthetic rate, incomplete autotrophy, incomplete rooting system, physiological disorders and etc (Kozai, T. and Iwanami, Y., 1988 b.). According to these phenomenon, very low growth and low survival rate of the plants could obtain from an in vitro condition and the acclimatization stage (Kozai, T. 1988).

The experiment on CO₂ enriched into culture vessels was conducted for the study on growth enhancement and autotrophic development of papaya plantlets in vitro.

MATERIALS AND METHODS

The explants preparation :-

Papaya lateral buds-derived plantlets variety of 'Ko Ko' were used in this experiment. Multiplication of the explants by culturing them on modified MS medium (1962) containing 100 mg/l myo-inositol, 2 mg/l glycine, 80 mg/l adenine sulfate, 30 g/l sucrose, 15% (V/V) coconut water, 0.1 mg/l naphthalene acetic acid (NAA) at pH 5.8 and 7.5 g/l agar (Burikam et al, 1989). The cultures were kept at 27°C under 2,000 lux light intensity, 16 hrs. photoperiod.

Effects of CO₂, sucrose and bottle caps on growth of papaya plantlets.

Papaya plantlets as described above were then cut into 1 cm long shoots. Each shoot was cultured under different conditions :-

- 1) Various concentrations of CO₂ gas supply into CO₂ incubator (Isuzu model IP-31) : 0.03 (atmosphere), 1, 3 and 5% CO₂
- 2) Various concentrations of sucrose added to the media : 0, 1, 2 and 3% sucrose
- 3) Two types of bottle caps : plastic and aluminum foil with membrane filter for gas exchange.

These shoots were cultured in CO₂ incubator at 28-30°C under 1,200 lux light intensity, 16 hrs. photoperiod for three weeks.

Fresh and dry weights of papaya shoots at day 0 and day 21 were recorded. Dry weight of these shoots was obtained after subjecting the explants in the vacuum oven at 70°C, 15 lbs/inches² Psi for 24 hours. Final concentration of CO₂ gas inside the culture bottles was evaluated by the gas chromatography (Shimadzu model GC-R1A). All data were analysed by Statistical Analysis System or SAS (SAS Institute, 1988). Final weight of papaya plantlets was adjusted to the beginning weights by the Covariance method and the Leastsquares means were used to test for significant differences of the average values.

RESULTS AND DISCUSSIONS

There was no significant difference of the average fresh weight of papaya plantlets cultured in various CO₂ concentrations ($P = 0.2147$; d.f. = 3), but the average dry weight was significant difference ($P = 0.0245$; d.f. = 3) as shown in Table 1. Table 2 shows that the fresh and dry weight of papaya plantlets cultured in bottles covered with plastic caps and aluminum foil were significant difference (fresh weight $P = 0.0144$; dry weight $P = 0.0018$). At day 21, CO₂ concentrations inside bottles covered with plastic caps were greater than those of the aluminum foil (Figure 1). Figures 2 and 3 show the comparisons on increasing percentages between fresh and dry weight

Table 1 The average weight of three-week-old papaya plantlets cultured in CO₂ enrichment chamber.

CO ₂ concentration (%)	Weight (gm)*	
	Fresh	Dry
0.03 (atmosphere)	0.0927 a	0.0096 ab
1	0.1087 a	0.1080 a
3	0.1202 a	0.0120 a
5	0.1470 a	0.0083 b

* Numbers in a column followed by the same letter are not significantly different ($P > 0.05$; LS means procedure).

Table 2 The average weight of three-week-old papaya plantlets cultured in bottles covered with aluminum foil or plastic lids.

Type of lids	Weight (gm)*	
	Fresh	Dry
aluminum foil	0.0930 a	0.0087 a
plastic lid	0.1413 b	0.0117 b

* Numbers in a column followed by the same letter are not significantly different ($P > 0.05$; LS means procedure).

of papaya plantlets cultured on MS media with different sucrose concentrations (0, 1, 2 and 3%), and enriched CO₂ concentrations at atmosphere (0.03), 1, 3 and 5%

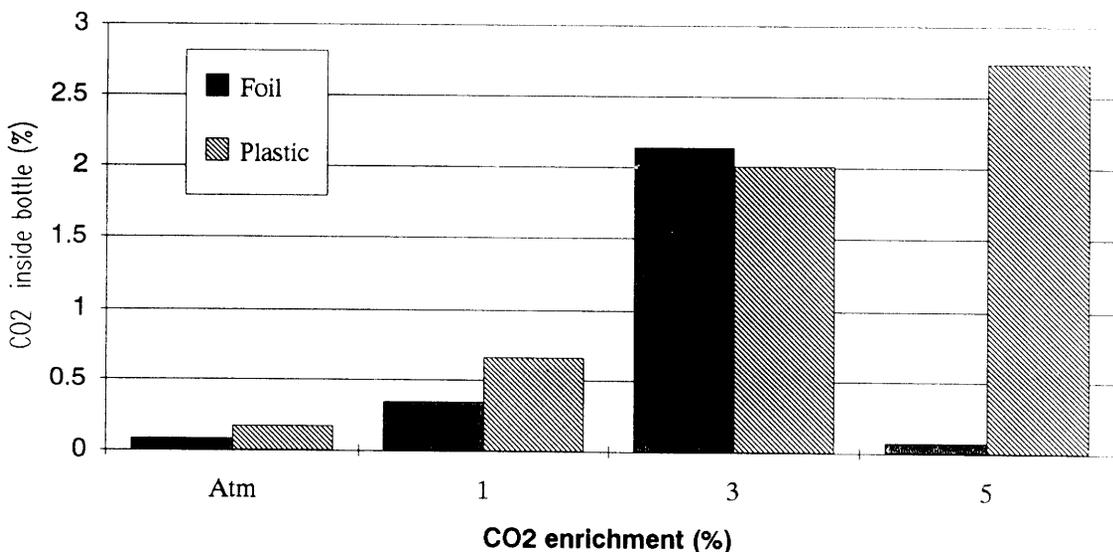


Figure 1 Concentrations of CO₂ inside the bottles covered with aluminum foil and plastic

Figure 2.

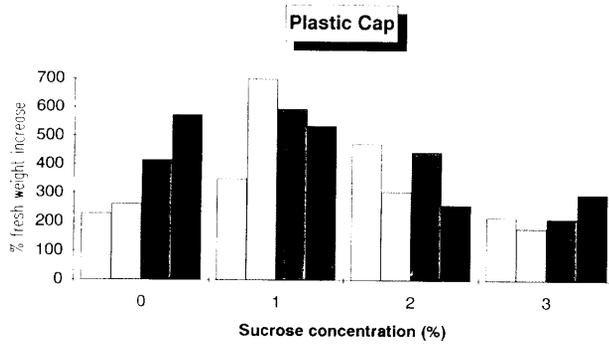
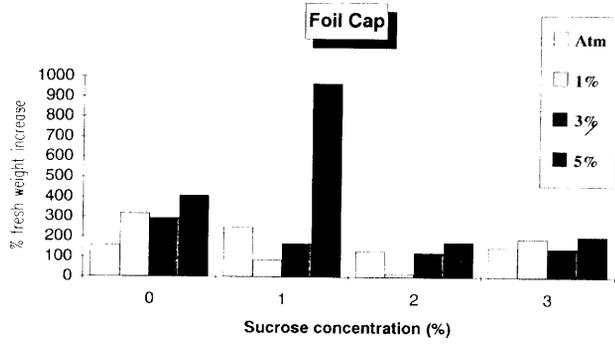


Figure 3.

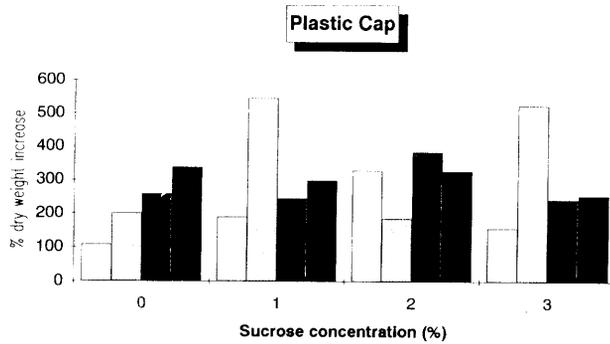
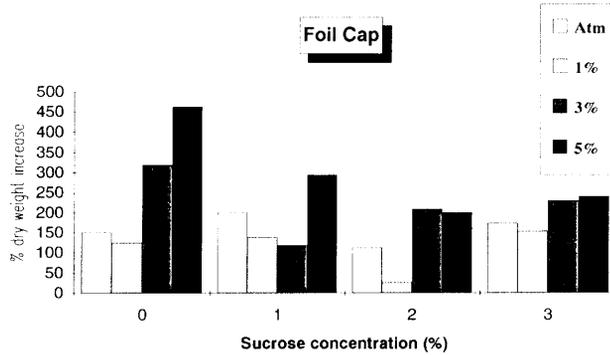


Figure 2 - 3 Fresh weight (figures 2) and dry weight (figure 3) of three-week-old papaya plantlets cultured in the media containing sucrose at different concentrations of CO₂

in bottles covered with plastic caps or aluminum foil.

After three weeks in culture, the highest increasing percentage of fresh weight of papaya shoots was obtained from shoots cultured in MS medium containing 1% sucrose in 5% CO₂ enrichment and covered with aluminum foil. The highest increasing percentage of dry weight was obtained from shoots cultured in the medium containing 1% sucrose in 1% CO₂ enrichment and covered with plastic caps. However, plants naturally have limited range in using CO₂ for photosynthetic system. Excess CO₂ in atmosphere may decrease photosynthetic rate or cause physiological disorder in plants (Kozai, T., Oki, H., and Fujiwara, K. 1988 a). Kozai and Iwanami (1988) found that when CO₂ was enriched to carnation plantlets in culture at concentrations of 0.1% - 0.15%, the percent increase of carnation plantlets was observed when sucrose was added to the media, especially at 1%.

CONCLUSIONS

Comparative results on growth of papaya shoots cultured in varying concentrations of CO₂ and sucrose, and varying types of bottle covered indicated that the increasing percentage of fresh weight was highest when papaya shoots were cultured in MS medium containing 1% sucrose, 5% CO₂ enrichment, bottle covered with aluminum foil. Papaya shoots cultured in MS medium with 1% sucrose, 1% CO₂ enrichment in bottle covered with plastic caps yielded the highest increasing percentage of dry weight. By statistical analysis, the percent increase of fresh weight was not significant difference at any concentrations of CO₂ enrichment but the difference was found with dry weight.

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