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Effects of Plant Growth Regulators on Papayas *(Carica papaya)* Cultured *in vitro*

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ABSTRACT

Micropropagation of papaya variety "Khag Dum" and "Solo" was obtained by culturing papaya shoot apices and buds on modified MS medium containing 0.5 mg/1 BA and 0.2 mg/1 NAA. The increasing rate of multiple shoots was about 7 folds of plants during subculturing periods. The papaya plantlets approximately 2-3 cm. in height could be rooted on MS medium with 1.5 mg/1 IBA. After subculturing these shoots onto the same medium more than one year, the vitrification of shoots and plantlets was observed. Further multiplication and rooting of vitrified papaya shoots and plantlets was unsuccessful. Therefore, interchangeable of the media containing with 0-5 mg/1 kinetin, 15% coconut water, and 0-0.2 mg/1 NAA were substituted for subculturing. Papaya plantlets were best grown on MS medium containing 15% coconut water, 0.1 mg/1 NAA, and 120 mg/1 adenine sulfate. About 80% of these plantlets were successfully rooted on MS medium with 1.5 mg/1 IBA.

INTRODUCTION

Papayas have conventionally been propagated from seeds, which considerable variation usually exist in commercial plantings. In order to retain selected characteristics, other methods such as clonal propagation via tissue culture technique would be more favorable. However, clonal propagation such as grafting and root cuttings are impractical when carry out on a large scale. Therefore, tissue culture technique will offer a valuable alternative in papaya propagation. This method can produce many true-to-type papaya seedlings or seedlings with special characteristics in a short period of time.

Litz and Conover (1978) succeeded on papaya micropropagation from shoot apices and lateral buds. Burikam *et al.* (1987) repeated the experiment on two papaya varieties; Khag Dum and Solo. About 7 folds increased in plant number at every subculturing period was obtained, within one year the vitrified plantlets were observed and unable to maintain the culture. However, the vitrified plants might resulted from the prolong exposure to benzyladenine in the media (John 1986). Prevention of vitrification can be obtained by (i) increasing the agar concentration (Debergh *et al.* 1981), (ii) periodically substituted of benzyladenine with an alternative cytokinin N_6-(isopentyl)-adenine (John 1986), and (iii) storing the culture in cold temperature at 3-4 °C for certain periods can restore vitrified cultures to normal growth (Rugini and Verma 1983). In this experiment, both kinetin and coconut water in the media for benzyladenine substitution, and the increment of agar concentration were used to solve vitrification problem.

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MATERIALS AND METHODS

Papaya shoot apices and lateral buds of variety "Khag Dum" and "Solo" were surfaced sterilized by 10% Clorox with 0.1% Tween 20 for 20-30 minutes. Following three rinses in sterile, distilled water, the explants were put onto MS media containing with 1 mg/1 kinetin or 1 mg/1 kinetin and 0.1 mg/1 NAA (Murashige and Skoog 1962). The mixture were solidified with 7 mg/1 agar and the pH was adjusted to 5.8. The cultures were incubated in a growth room at 28 °C for 16 hours photoperiod. After 2 months in culture, the enlarged explants were subcultured onto modified MS media containing 120 mg/1 adenine sulfate, 0.5 mg/1 BA and 0,2 mg/1 NAA for multiple shoot induction. Thereafter, the cultures were subcultured every three weeks onto the second medium. When the cultures appeared to be vitrified, 15% coconut water and 0-5 mg/1 kinetin were used in substitution for benzyladenine in modified MS media with 120 mg/1 adenine sulfate and 8.5 g/1 agar. Normal papaya plantlets about 2-3 cm. high were individually transferred onto MS media containing 0-2 ppm. IBA and/or NAA for root induction.

RESULTS AND DISCUSSION

After subculturing papaya bud culture onto MS medium containing 0.5 mg/1 BA and 0.2 mg/1 NAA, about 80% of the plantlets, appeared to be vitrified within one year. The symptoms for vitrified papaya culture were translucent stems with thickened leaves, turgid and brittle with an abnormal glass-like appearance to the culture surfaces (Figure 1). Shoot multiplication and root induction from these vitrified plantlets were unable to obtain. Vitrification, whether spontaneous or induced, was associated with morphological, anatomical and physiological changes that resulted in accelerated growth and development. Kevers *et al.* (1984) suggested that cell wall changes due to enhanced peroxidase activity mediated through ethylene might be the mechanism by which vitrification occurred. Ethylene does appear in tightly stoppered flasks at concentrations that are sufficient to modify morphogenesis. Most of the effects of ethylene described are, however, inhibitor and inducing isodiametric cell growth causing swollen stem. The cytokinin N_6-(isopentyl)-adenine was successfully used as vitrification preventive, but it is very expensive. Therefore, kinetin and coconut water were used as benzyladenine substitution in this experiment. The effects of benzyladenine (BA), kinetin, NAA, and coconut water on papaya growth in the culture were shown in Table 1, 2, and Figure 2. Table 1 illustrated the effects of kinetin, BA, and NAA supplemented in modified MS media with 120 mg/1 adenine sulfate. The culture in the media supplemented with 0.5 mg/1 kinetin and 0.2 mg/1 NAA yielded extensive proliferation and vitrified shoots like those plants in the media containing 0.5 mg/1 BA and 0.2 mg/1 NAA. Table 2 showed the effects of kinetin and benzyladenine with NAA and 15% coconut water in modified MS media with 120 mg/1 adenine sulfate. Vitrification of shoot was disappeared in most combinations, but the extensive growth of plants in culture including proliferation were seen in the media with 0.1 mg/1 NAA without kinetin or benzyladenine. According to this experiment, normal growth of papaya plants in culture was obtained from media with neither kinetin nor benzyladenine. However, it is necessary to use modified MS media supplemented with 0.5 mg/1 BA and 0.2 NAA as shoot multiplication media, and modified MS media containing with 15% coconut water and 0.1 mg/1 NAA as an alternative in order to retain normal growth of papaya plantlets in the culture. Regardless of the causes of vitrification, it is the phenomenon that may be useful in the micropropagation of papaya because of its capacity to boost multiplication rates. The possible solution would be:

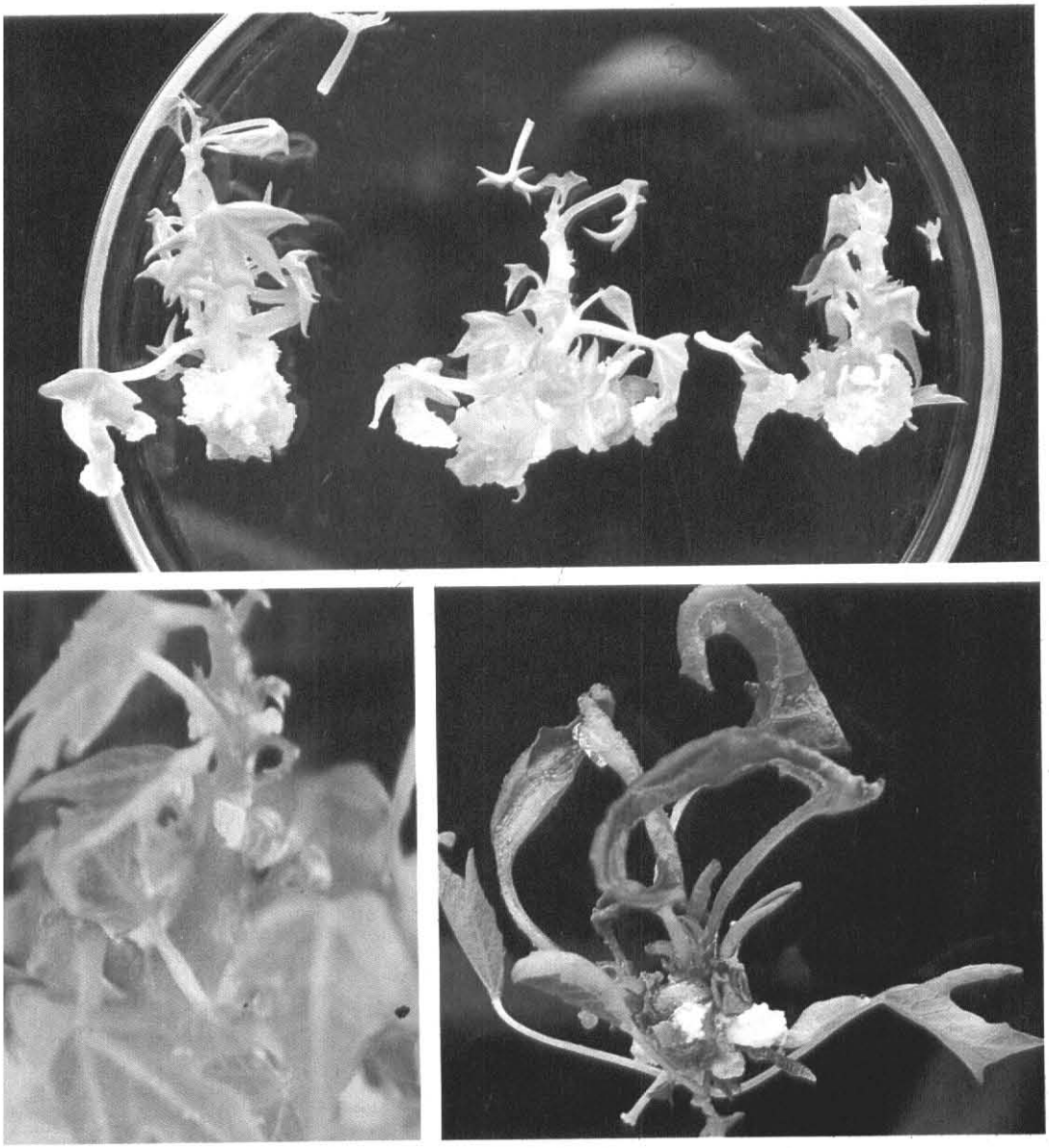

Figure 1 Symptoms of vitrification of papaya plantlets subcultured onto MS medium containing with 0.5 mg/1 BA and 0.2 mg/1 NAA.

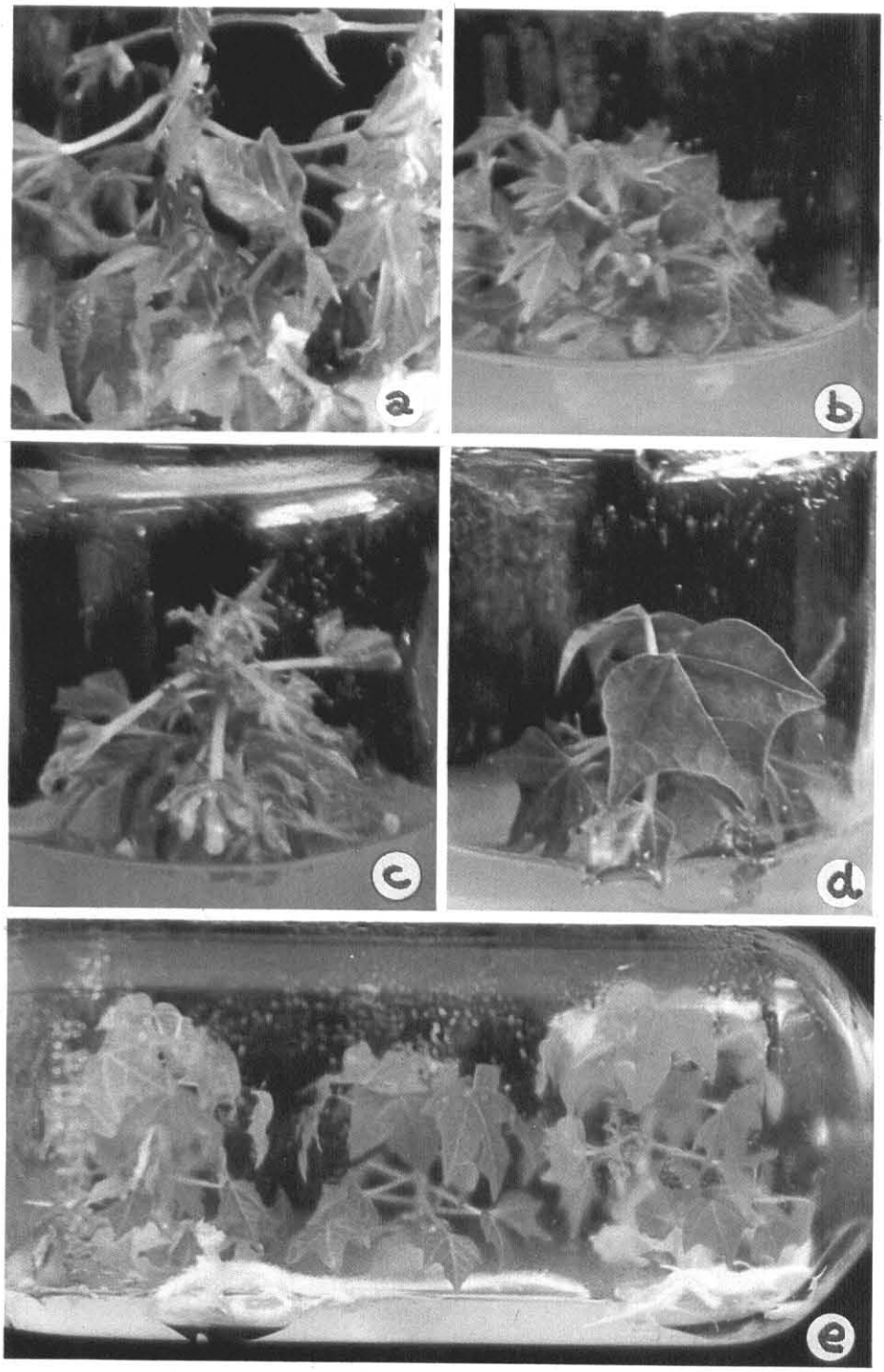

Figure 2 Growth of papaya plantlets subcultured onto differential modified MS media with 120 mg/1 adenine sulfate: (a) 0.5 mg/1 BA and 0.2 mg/1 NAA; (b) 15% coconut water with 0.5 mg/1 BA and 0.2 mg/1 NAA; (c) 0.5 mg/1 kinetin and 0.2 mg/1 NAA; (d) 15% coconut water with 0.5 mg/1 and 0.2 mg/1 NAA; (e) 15% coconut water with 0.1 mg/1 NAA.

Table 1 The effect of kinetin or BA with NAA on plant regeneration after 3 weeks*

NAA (mg/1)	Kinetin (mg/1)					BA (mg/1)	
	0	0.5	1	2	5	0	0.5
0	—	+,E	+,E	+,E	—	—	++,E
0.1	C	++	++	++	—	C	++,E
0.2	C	+++,C,V	++,C	++,C	—	C	+++,C,V

*basal medium is Murashige and Skoog with adenine sulfate 120 mg/1

— = no callus, no plant regeneration
\+ = regeneration, some proliferation
++ = regeneration, moderate proliferation
+++ = regeneration, extensive proliferation
C = callus
E = extensive growth of leaves
V = vitrified shoots

Table 2 The effect of kinetin or BA with NAA on plant regeneration after 3 weeks*

NAA (mg/1)	Kinetin (mg/1)					BA (mg/1)	
	0	0.5	1	2	5	0	0.5
0	E	+++	+	+	—	E	EC
0.1	+++,E	++,E	++,E	++,E	—	+++	++,E
0.2	++,C	++	++	++	—	++,C	+++,C

*basal medium is Murashige and Skoog with adenine sulfate 120 mg/1 and coconut water 15% V/V

— = no callus, no plant regeneration
\+ = regeneration, some proliferation
++ = regeneration, moderate proliferation
+++ = regeneration, extensive proliferation
C = callus
E = extensive growth of leaves
V = vitrified shoots

1. Establish primary cultures *in vitro* on a medium without growth substances
2. Remove water and allow vitrified growth to return to normal
3. Subculture and repeat.

In order to be successful and acceptable for clonal propagation, it will be necessary to demonstrate that: (a) the changes that occur during vitrification are physiological effects, not somaclonal variation; (b) the plantlets can revert to truly normal growth *in vitro;* and (c) the rooted plantlets are able to develop normally when transfer to non-sterile conditions in the soil.

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