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Determination of Cultivar and Sex of Papaya Tissues Derived from Tissue Culture

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ABSTRACT

One hundred milligrams of papaya tissues were used to identify the peroxidase isozyme pattern by using disc polyacrylamide gel electrophoresis. Results obtained from papaya tissues *in vitro* were the same as those of mature papaya plants. It is suggested that this technique is suitable for the selection of cultivar and sex of papaya cell lines for tissue culture propagation.

INTRODUCTION

Papaya is native plant of Central America but now is wildly grown in the tropical and sub-tropical regions. In Thailand, papaya can be grown throughout the country, especially in the provinces of Ratchaburi, Nakorn Pathom, Samut Sakorn and Nakorn Ratchasima. Papaya *(Carica papaya* L.*)* is a member of Family Caricaceae, Class Dicotyledon. There are three types of plant bodies; pistillate, staminate and hermaphroditic which can be differentiated only at the flowering time. (Mekako and Nakasone, 1975). The evidence of cultivar and sex determination in the early stage of papaya growth was still unknown.

The study of isozyme by electrophoresis has been a useful powerful tool for many investigators. The technology has been successfully employed in plant and culture plant cell, e.g. in studies related to genetic investigations and to the physiology of plant development, particularly in studies on differentiating process. (Wetter, 1978).

It is well known that vegetative propagation of papaya through conventional methods has not been successful. At present, mass propagation of papaya plants can be accomplished via tissue culture. (Litz and Conover, 1978). In this experiment, the polyacrylamide gel electrophoresis of cationic peroxidase isozyme has been used for determination of cultivar and sex type of papaya tissues derived from tissue culture.

MATERIALS AND METHODS

1. Papaya plant cultivars

   Solo : Hermaphroditic plant ; 3 strains
   - Cavite
   - Line 8
   - Line 2

   Khaegdam : hermaphroditic plant
   Khaegnuan : hermaphroditic plant
   Co.2 : pistillate and staminate plant

Leaf tissues of papaya plantlets derived from papaya buds cultured in MS medium containing with 0.5 ppm BA and 0.2 ppm NAA (Burikam *et. al.*, 1987) were used throughout this study.

The sample materials were immediately frozen in deep freezer at -40°C for later analysis.

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2. Manitol, 3% (v/v)
3. Extraction medium :
   Acetate buffer 0.1 M pH 4.0
4. Reagent for gel preparation : acidic buffer system (Reisfeld *et. al.* 1962)
   4.1 Stock A-22.4 ml acetic acid (glacial), 4.6 ml of TEMED and 48 ml of 1 N KOH to 100 ml of solution (pH 4.2)
   4.2 Stock B-40 g acrylamide and 1.0 g Bis in 100 ml of water.
   4.3 Stock C-140 mg ammonium persulfate in 100 ml of water.

   The above solutions were all stored at 4°C.
5. Reagent for electrophoresis (Reisfeld *et al.* 1962)

The electrode buffer for acidic separating gel consists of 17.25 g of glycine and 2.4 g of glacial acetic acid in 1 litre of stock solution (pH 4.0)

Dilute the buffer once only with distilled water ratio 1:1.

6. Gel detection reagent : cationic peroxidase isozyme
   6.1 Stock A - Dissolve 420 mg 3-Amino 9-Ethylcarbazole and 290 mg B-naphthol in 200 ml of acetone
   6.2 Stock B -Dissolve 3.78 Tris and 4.05 ml acetic acid in 2.5 litres of water (pH 4.0)
   6.3 Stock C-3 % H_2O_2 (v/v)
   Before staining, mix stock A : Stock B Stock C ratio 20:80:1

PROCEDURES (Sriprasertsak, 1988)

1. Extraction
   1.1 Collect leaf tissues of papaya tissue culture and wash with 3% manitol solution
   1.2 0.1 g of samples were ground in a chilled tissue grinder with 100 ul of extract buffer.
   1.3 The crude extract was centrifuged at 15,000 rpm for 10 minutes at 0°C.
   1.4 The supernatant was then mixed with 5% glycerol (v/v) and stored at -20°C.
2. Enzyme Separation
   2.1 Preparation of gels
      a. Select 10 glass tubes (9 by 0.5 cm inside diameter)
      b. Prepare the separating gel (10%) by mixing together 3 ml of stock A, 6 ml of stock B, 3 ml of distilled water and 12 ml of stock C
      c. De-gas with degasser for 2 mins.
      d. Immediately fill the tubes with above solution to the 8 cm mark with Pasteur pipette.
      e. Layer top of gel with water using a Pasteur pipette. Do not disturb the surface solution.
      f. Allow for at least 2 hours before using.
   2.2 Electrophoresis
      a. Wash surface of gel with electrode buffer
      b. The tubes containing gel were placed in the grommets in the upper chamber of the electrophoresis apparatus.
      c. Enough electrode buffer was added to the lower and upper chamber so that the tubes can make contact with.
      d. Place enzyme extract on the top of the gel under the upper chamber, about 10-15 μl.
      e. The isozymes were separated from anode (+) to cathode

(−) at 2 mA/tube constant current.

f. Electrophoresis was terminated after 1.5-2.0 hours, when the marker dye front had migrated with 1 cm of the bottom of the gel.

Remark: In this experiment, the bromophenol blue cannot be used as marker dye because of its colour turning to light-yellow at acidic buffer system. For calculation the Rf relative mobility of zymogram banding pattern, the leaf pigment of crude extract found suitable for this purpose.

g. The tubes were removed from the apparatus and the gel were excised by rimming method.

3. Enzyme detection

3.1 Transfer the gel to 1.5 by 6.0 cm test tubes which contain the peroxidase staining solution.

3.2 Allow to stand for 20-30 minutes in the dark at room temperature.

3.3 The staining solution is discarded when development is judged completely. The gels are rinsed and stored in 7% acetic acid (v/v).

4. Recording of results

The zymogram banding patterns of cationic peroxidase isozymes are recorded by drawing the stained gel on white paper. From this it is possible to determine the Rf relative mobility by calculating,

$$Rf = \frac{\text{the distance migrated by isozyme band}}{\text{the distance migrate by leaf pigment}}$$

RESULTS AND DISCUSSION

Preliminary tests

The cationic peroxidase isozyme was investigated for determination of cultivar and sex of papaya tissue derived from tissue culture by using electrophoresis technique. Preliminary study showed that leaf tissues of 5-6 month old plantlet in step II of papaya tissue culture (Figure 1) were suitable for isozyme analysis. Only one hundred milligrams of leaf tissue were sufficient for enzyme extraction. Electrophoresis was carried out using 40 μl of crude extract and the appropriate conditions for enzyme separation consisted of running the extract from anode to cathode at 2 mA/tube for 90-100 minutes under a temperature range of 5-7°C.

Comparison of cationic peroxidase isozyme

In total, 9 bands were detected and numbered from 1 to 9 according to their Rf relative mobility. It is found that the band number 1, 5, 6, 8 and 9 are present in all cultivars examined. It appeared that zymogram patterns of cationic peroxidase isozyme can be used to differentiate among the strains of hermaphroditic plant. Cavite, Line 8 and Line 2 of Solo papaya can be identified by the band No. 2, 3 and 4 (Figure 2). However, no differences in zymogram pattern between Line 2, Khaegdam and Khaegnuan cultivars (Figure 3). It is cleared, however, that cationic peroxidase isozyme can differentiate the sex of papaya tissue culture plant. The staminate (♂) and pistillate (♀) plantlets of Co. 2 cultivar have been identified by the occuring of band No. 2, 4 and 7 in the staminate while absent in the pistillate. (Figure 4)

Zymograms of cationic peroxidase isozyme are proved to be useful for determination of cultivar and sex of papaya tissue culture. Although this results can not show clear cut different in all cultivars studies. It is important to look at many

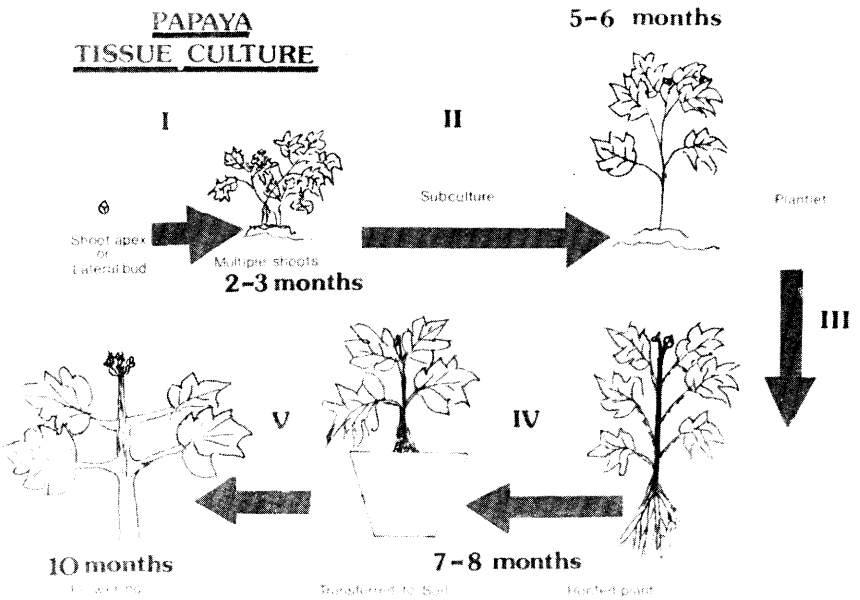

Figure 1 Schematic of papaya tissue culture from lateral bud to flowering stage.

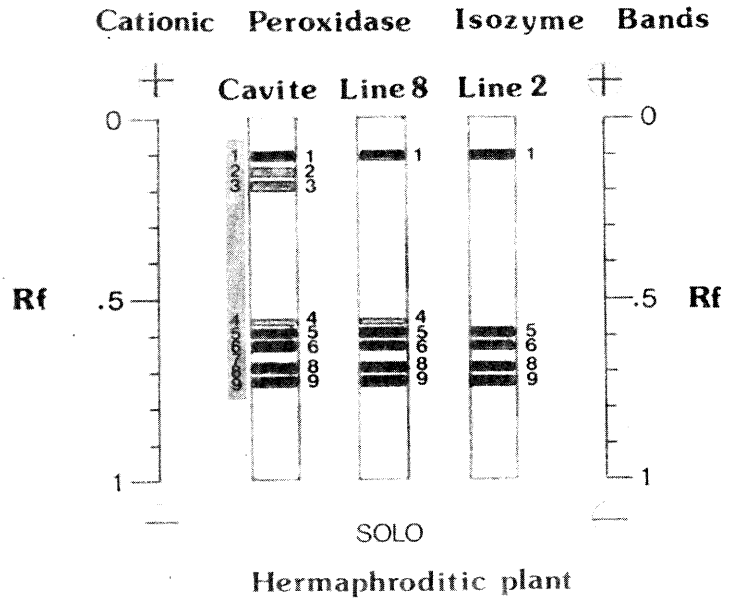

Figure 2 Cationic peroxidase isozymes of hermaphroditic plant of three Solo papaya strains.

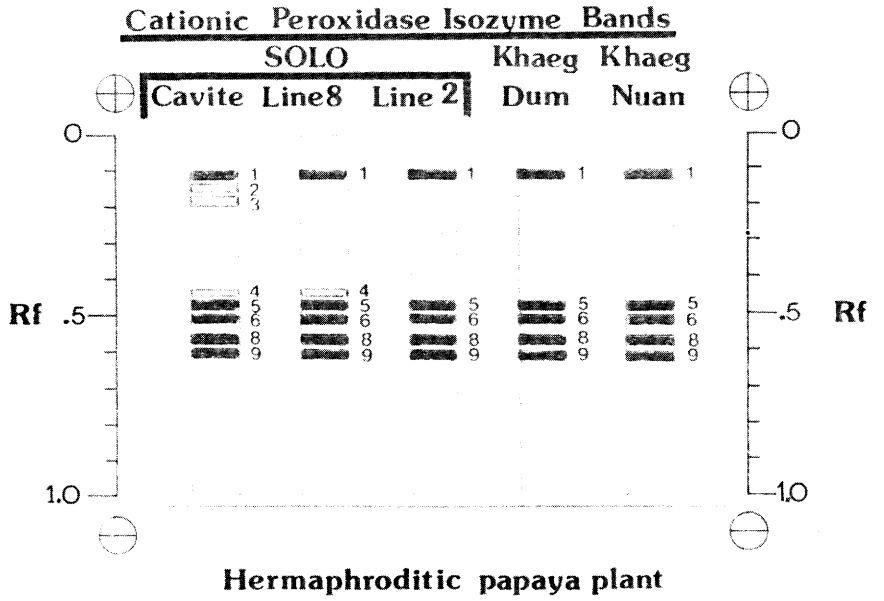

Figure 3 Cationic peroxidase isozymes of hermaphroditic plant of Solo, Khaegdam and Khaegnuan papaya.

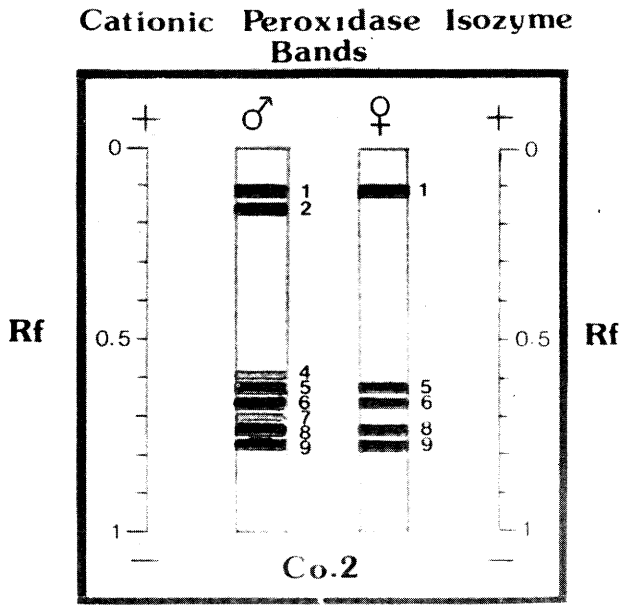

Figure 4 Cationic peroxidase isozymes of staminate (♂) and pistillate (♀) plant of Co. 2 papaya.

different exzyme systems for complete identification. For example, Wetter and Kao (1976) could identify calli from the somatic fusion of *Nicotiana glauca* Grah. and *N. langsdorffii* Weinm. by the zymograms obtained from lactate and alcohol dehydrogenase and aminopeptidase.

Cationic peroxidase isozyme of leaf tissues plays important role in characterizing the sex of papaya tissue culture plant. Generally, the differentiation of papaya sexuality, whether they are pistillate, staminate or hermaphroditic plant, can be examined at the mature stage by their morphological characteristics (Mekako and Nakasone, 1975). In this paper, electrophoresis of cationic peroxidase isozyme showed a successful method in distinguishing the sexual of papaya at the early stage of plant age. It is suggested that this technique is suitable for the selection of cultivar and sex type of papaya cell lines for mass propagation of papaya via tissue culture.

However, further study should be done to confirm that no variation occur when mature papaya plant derived from the same planted are further tested.

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