

result of the acquisition amount of cell isolation and purification processes are presented in table 1 and 2.

TABLE 1
THE NUMBER OF CELLS

E0	E1	E2	E3
1,56.10 ⁶ e	5,89.10 ⁶ d	7,41.10 ⁶ b	1,34.10 ⁷ a
8,42.10 ⁵ e	1,34.10 ⁷ a	1,25.10 ⁷ a	1,49.10 ⁷ a
5,48.10 ⁵ e	5,04.10 ⁶ cd	5,41.10 ⁶ cd	7,92.10 ⁶ bc

TABLE 2
CELLS VIABILITY

E0	E1	E2	E3
96.3 a	98.91 a	97.58 a	95.65 b
100 a	100 a	99.31 a	99.61 a
100 a	100 a	100 a	95.53 a

and of tissue or organ in nearly all living plants. Usually the most commonly used is the leaf (Wetter, 1988). This is because the cells produced are uniform in size. The leaves are young and actively growing. Callus give better results. One important factor in the acquisition of explant cells are the source and the growth conditions, which include explant age, time of harvest and use of organs or tissue (Wetter and Dixon, 1994). The release of cells from the tissue is depending on the type and concentration of the enzyme. Pektinase to digest the middle lamella, whereas cellulase and hemiselulase to digest the cell wall (Wetter, 1988). Pektinase enzyme that is often used is Macerozyme pectinase R-10 [6]. to isolate leaf protoplasts of peach (*Prunus persica*) by using enzyme Onozuka R-10 (2%) combined with cellulase (0.5%) with cell viability reached 90% for the leaf and 60% for the cell. Dixon (1987) using cellulase (*Rhizopus polygalacturonase*) to isolate the leaf mesophyll cells and the concentration used is 0.5% / L with incubation time for 20 minutes. The yield of cells from other cells varies for each plant species. Some species are tolerant of the long incubation time but there protoplasts cells damaged by long incubation [7]. Enzyme activity is highly dependent on pH. Generally, the optimal pH of enzyme activity is 5.4 to 6.2. In an experiment conducted by Dixon (1987) used pH is 6.0 in the medium maceration. Dixon (1987), optimal pH for the enzyme cellulase 5-6 and for the enzyme Onozuka is 4-5. During the isolation of

common pH changes that could affect the enzyme activity. In order to maintain the pH during the process progresses, the enzyme solution added buffer compounds such as MgSO₄ (Gambor and Dixon, 1981), MES (N-Morpholino Ethane sulphonic acid) (Dixon, 1987). The method of incubation there are two kinds of methods using non-agitation and agitation. Agitation method using penggojog (*shaker*) with a certain speed. [3]. using a magnetic stirrer with a speed that is not too fast. There is also a method of shaking in which the explants were incubated in a shaker for a certain time [9]. Giving osmoticum to maintain osmotic pressure cell. Osmoticum commonly used are sorbitol, glucose [10]. In an experiment organic salts used as an osmoticum such as CaCl₂ .2 H₂ O.

3. Cell Growth

1. Cell Growth Medium

After the cells were isolated, then the number of cells counted and then cultured in growth medium. The first step in cell cultivation conducted in Petri dishes (Fig.2)



Fig. 2. Mesophyll cell culture *C. asiatica* suspension after one day after seeding in Petri dishes with MS medium.

This is done because the acquisition of cells from the cell isolation process is only sufficient for the volume of approximately 25 ml of culture in the medium. When placed in Erlenmeyer not meet the initial density of planting that is 10⁵ / ml. However, at this stage can be observed the growth of the cells by observing the cell populations in a haemocytometer. From the observation obtained by the observation that, two days after planting of cells, almost all cells still looked viable, the cells were grouped or individually. Besides, the density of cells in the haemocytometer is increasingly crowded. On the fourth day there appeared to start cell groups