

## Effects of different enzyme combination and dissociation-time on the protoplast isolation of alfalfa

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**Introduction** At present, as donor of protoplast dissociation is different to alfalfa, some factors have influence on protoplast dissociation, such as the kinds of enzyme, the concentration and dissociation-time. Using hypocotyl of alfalfa as tested materials, by setting different the kinds of enzyme, the concentration and combination of enzyme, dissociation-time in this study in order to provide theory and practical guidance for extension of alfalfa biotechnology.

**Materials and methods** According to the routine methods for inducement cultivating asepsis seedling. After asepsis seedling was cultivated 10d, the hypocotyls were chopped.

Usually 1.0 g chopped hypocotyls in a CPW-13 solution (Frearson et al., 1973) were incubated in the dark at 25°C for 1h then submitted to 10 ml filter-sterilized enzyme solution I. The same method was treated in the enzyme solution II, III, IV as the enzyme mixtures I. Chopped hypocotyls were shaken at 60 rpm for 6h in the dark.

Usually 1.0 g chopped hypocotyls in a CPW-13 solution were incubated in the dark at 25°C for 1h then submitted to 10 ml enzyme solution IV. Chopped hypocotyls were shaken at 60 rpm 25°C for 4h, 6h, 8h and 10h in the dark.

The mixture was passed through a nylon sieve (38.5 μm pore sizes) and 15 ml of CPW9M solution was added. The protoplasts were collected by centrifugation for 5 min at 60 rpm and resuspended in the washing solution. The washing treatment was done twice. Protoplasts, free of debris were carefully removed from the interface of the solutions, and protoplasts were rinsed twice with 15 ml of KM8p medium (Kao and Michayluk, 1975). A small sample of protoplasts in the washing solution was stained with 0.01% (w/v) phenosafranin. The yield of protoplasts was determined with a Malassez counting chamber.

**Results and discussion** Even conspecific plant, initiative materials are different, there are different to concentration of enzyme and dissociation-time. Chopped hypocotyls were treated in four enzyme solutions for 6h, it was better the condition of protoplast dissociation in enzyme solution II in this experimentation. In the same condition of protoplast dissociation, protoplast dissociation production gradual increase along with protraction of dissociation-time, but dissociation-time is overlong will result in sharp increase of cell debris it will affect protoplast culture and prior dissociative protoplast is able to occur fracture. It was better dissociation-time that the hypocotyls were treated for 6h, at the same time cell debris was less.

**Table 1** The concentration and combination of enzyme.

Name	Celulose (%)	Pectinose (%)	maceroryme R-10 (%)
enzyme solution I	1	0.8	0
enzyme solution II	1	0.8	0.5
enzyme solution III	2	0.8	0
enzyme solution IV	2	0.8	0.5

**Table 2** The effects of different enzyme combination of alfalfa hypocotyls on protoplast dissociation for 6h.

Name	yield of viable protoplast (10 <sup>6</sup> /ml)	cell leakage
enzyme solution I	0.26	large amount
enzyme solution II	0.95	small
enzyme solution III	0.31	quaterare
enzyme solution IV	0.40	large amount

**Table 3** The effects of different enzyme dissociation-time of alfalfa hypocotyls on protoplast dissociation in enzyme solution II.

dissociation-time (h)	yield (1 × 10 <sup>6</sup> g <sup>-1</sup> )	visibilty (%)	yield of viable protoplast (1 × 10 <sup>6</sup> g <sup>-1</sup> )	cell leakage
4	0.22	7920	0.33	quaterare
6	0.96	8690	1.09	small
8	1.56	8110	0.74	small
10	1.01	9020	0.56	large amount

### Reference

Wang Hai-bo, Yu Yong-xiong. (2006). The Research of Protoplast Dissociate Conditions of Alfalfa [J]. *Chinese Journal of Grassland*, 28(5): 33-37.