Effects of Different Enzyme Combination and Dissociation-Time on the Protoplast Isolation of Alfalfa

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Key words : alfalfa ,the kinds of enzyme ,concentration of enzyme ,dissociation-time ,protoplast

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℃ 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℃ for 1h then submitted to 10 ml enzyme solution Ⅳ ,Chopped hypocotyls were shaken at 60 rpm ,25℃ 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) phenosafranine .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 corcertration and combintin of ensyme .*

Name	Ceihilose (%)	Pectinose (%)	macereryme R-10(%)
enryme soltion Ⅰ	1	0 .8	0
enryme soltion Ⅱ	1	0 .8	0 .5
enryme soltion Ⅲ	2	0 .8	0
enryme soltion Ⅳ	2	0 .8	0 .5

Table 2 *The effects of different enzyme combintion of alfalfa hypocotyk coprotoplast dissociation for on .*

Name	yield of vinble protoplast (10^6/ml)	cellbreabage
enryme soltion Ⅰ	0 .26	large amount
enryme soltion Ⅱ	0 .95	small
enryme soltion Ⅲ	0 .31	qutерare
enryme soltion Ⅳ	0 .40	large amount

Table 3 *The effects of different enzyme dissociation-time of alfalfa hypowtyls onprotoplast dissociztion in enryme solition Ⅱ .*

dissociotion-time(h)	yield($1\times10^6 g^{-1}$)	visbility(%)	yield of vinble protoplast($1\times10^6 g^{-1}$)	cell brealage
4	0 .22	7920	0 .33	qutерare
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 .