Effects of different factors on the hypocotyls protoplast isolation of common sainfoin

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Somatic hybridization which based on protoplast culture has been widely used in forage breeding . In this text , we explore the technology of protoplast isolation , and hope to culture a new pasture cultivars with superior character .

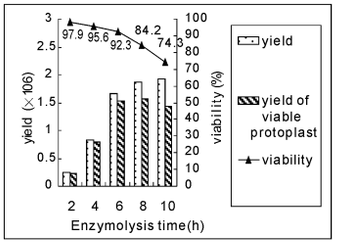
Materials and methods Axenic hypocotyl cultures of common sainfoin . Sainfoin seeds were surface-sterilized with 70% (v/v) ethanol (30s) , then transferred to 0 .1% (w/v) mercuric chloride (15min) , and washed 5 times with sterilized water . Seeds were put on half-strength MS basal medium (Murashige & Skoog , 1962) with 0 .7% (w/v) agar and 3% (w/v) sucrose . and kept in darkness(25±2℃) for 3-4d days .

They were cut transversely into slice (0 .5 mm wide approx .) When the hypocotyls were 2-3cm . The 1-g hypocotyls segments were treated (1 h) in 10 ml CPW salts solution containing 0 .7 M mannitol . ,then transferred into 10 ml filter-sterilized enzyme solution . After 2-10h incubation in the dark (25±2℃) , with gentle shaking (40 rpm) on a
rotary shaker , the mixture was passed through a nylon sieve(38 .5μm pore sizes) and 15 ml of CPW9M solution was added . The protoplasts were collected by centrifugation (100×g , 5 min) 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 and yield determined using a haemacytometer .

Results and discussion The principle of enzymolysis is getting viable protoplasts with lower concentration of enzymes and shouter enzymolysis time . Mannitol can adjust osmosis pressure of cell in protoplast isolation . If osmosis pressure too high or low ,cell membrane will ruptured . pH not only affect the viability of protoplast , but also the activity of enzymolysis . The result showed that the protoplasts with higher yield and quality were obtained by treating the hypocotyls with an enzyme mixture (pH5 .8) containing 2% cellulose Onozuka R-10+0 .5% Pectinase+0 .3% macerozyme R-10 and 0 .55mol/L mannitol for 6h .

Table 1 *The effects of different enzyme combination on protoplast isolation .*

Cellulase Onozuka R-10(g/l)	Pectin-ase (g/l)	Macerozy-me R-10(g/l)	yield of viable protoplast (1×10^6)
10	5	3	0 .83
10	5	5	1 .18
10	8	3	1 .54
10	8	5	1 .87
20	5	3	3 .20
20	5	5	3 .11
20	8	3	2 .34
20	8	5	1 .96



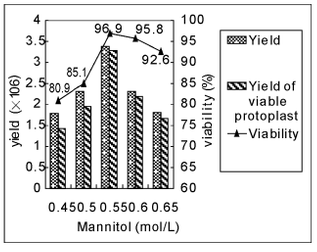


Figure 1 and Figure 2 *The effects of enzymolysis time and mannitol concentration on protoplast isolation .*

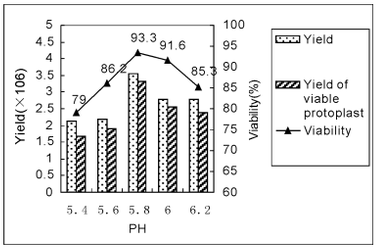


Figure 3 *The effects of pH on rotoplast isolation .*

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