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KEY WORD : PLANT REGENERATION, PROTOPLAST, SOYBEAN

PHONGYUTH NUALBUNRUANG : PLANT REGENERATION FROM CALLUS AND PROTOPLAST OF SOYBEAN (Glycine max (L.) Merr.) THESIS ADVISOR : ASSO. PROF. SANHA PANICHAJAKUL, Ph.D., ASSI. PROF. WANCHAI DE-EKNAMKUL, Ph.D. 187 pp. ISBN 974-581-696-5

The procedures for plant regeneration from primary leaf and hypocotyl callus of soybean (Glycine max) cv. SJ4 were developed. The conditions for callus induction from the primary leaf were on B5 medium supplemented with 1-3 mg/l BA, 0.10-0.20 mg/l NAA and 1,650 mg/l  $\text{NH}_4\text{NO}_3$ . The optimum medium for regenerating primary leaf callus were B5 medium supplemented with 1 mg/l BA, 0.15 mg/l NAA, 1,650 mg/l  $\text{NH}_4\text{NO}_3$  and the same medium with 2 mg/l BA, 0.15 mg NAA and 1,650 mg/l  $\text{NH}_4\text{NO}_3$ . For the hypocotyl, a piece located 2 mm lower from the cotyledonary node can be regenerated. The condition for callus induction from hypocotyl were on RV-5 medium supplemented with 0.1-0.4 mg/l IAA and 0.5-3 mg/l BA. The appropriate medium for regenerating hypocotyl callus was RV-5 medium supplemented with 0.5 mg/l BA and 0.2 mg/l IAA.

For the protoplasts isolation from callus, only friable callus which was cultured on B5 medium under the presence of 2,4-D and BA can be used as source. The optimum conditions for protoplasts isolation from callus were demonstrated by using 4% Cellulase R10, 0.5% Macerozyme R10 in 11 and 13% mannitol at pH 5.6. Appropriate incubation time was found at 2 hour in the dark on the rotary shaker at 100 rpm. The addition of 10 mM  $\text{CaCl}_2$  increased the yield of protoplasts. Three percents Cellulase R10 and 0.5% Macerozyme in 10% mannitol at pH 5.2 were found to be the proper combination for the isolation of protoplasts from intact cells growing in liquid culture. BA and 2,4-D were proved to be essential components for the cell lines used in protoplasts isolation.

The purified protoplasts obtained were cultured in K8P liquid medium free hormone and K8P liquid medium containing various combination of 2,4-D, BA, NAA and zeatin. The condition for regenerating cell wall was studied and illustrated. Cells regenerating from protoplast started to divide in four days after cultivated in the proper conditions.

Callus cultivated for over 12-15 months on the established medium supplemented with 2,4-D and BA can be regenerated in B5 medium supplemented with 0.15 mg/l NAA and 1 mg/l BA.