# B-49 : HIGH FREQUENCY PLANT REGENERATION FROM PROTOPLASTS OF CULTIVATED AND SOME WILD SPECIES OF *Medicago*

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Success in plant regeneration from cultured protoplasts, isolated directly from explant tissue or cell suspensions, has been very limited with *Medicago sativa* (c.v. Europe) and *M. falcata.* So far no reports are available on protoplast culture of *M. borealis* and *M. dzhawakhetica*.

An enzyme mixture containing Rhozyme (2% w/v), Meicelase-P (2% w/v), Macerozyme (0.03% w/v) and Driselae (0.5% w/v) was used to release viable protoplasts from cell suspensions of all the 4 species of *Medicago.* The cultural conditions were first optimized in relation to cell division and colony formation from protoplasts isolated from cell suspensions. Thereafter, these conditions were extended, with suitable modifications, to achieve embryogenesis from the cultured protoplasts of *M. dzhawakhetica, M. borealis, M. falcata* and *M. sativa*, with high frequency plant regeneration from cell suspension protoplasts of the latter 3 species.

Liquid or solidified (1.2% w/v agarose) medium based on Kao (1977) was used for culturing protoplasts. The final plating density of protoplasts was $5 \times 10^4$. During culture (50 - 60 days) the osmolarity of the medium of each culture dish was progressively reduced (every 10 days) by adding cell culture media as appropriate. These cell culture media were based on Murashige and Skoog (1962) medium (MS).

Plants could be established from the embryoids (2.5 mm) except from *M. dzhawakhetica* when cultured on MS based medium (agar 0.8% w/v) overlaid with 0.5 - 1 ml of modified Uchimiya and Murashige (1974) medium liquid.