B-35 : SOMATIC HYBRIDIZATION OF *Medicago sativa* (CVEUROPE) AND *M. falcata*

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Protoplasts isolated from cell suspensions of *Medicago sativa* (cv. Europe) and *M. falcata* (both tetraploids) were fused using a solution containing polyethylene glycol (PEG). After elution of PEG solution with a Ca^{+2} - free hypotonic solution the fused protoplasts (heterokaryon) were isolated using a micromanipulator and cultured in modified Kao (1977) medium with nurse protoplasts of *M. sativa*. During culture, (50 - 60 days) the osmolarity of the liquid nurse culture was progressively reduced (every 10 days), by adding cell culture media as appropriate. The cell culture media were based on Murashige and Skoog (1962) medium. Only 705 embryoids (2 - 3 mm) formed from the heterokaryons were incubated (26 C) in MS medium (agar 0.8 % w/v) overlaid with modified UM (Uchimiya and Murashige 1974) medium (1 ml liquid). From the cultured embryos, only 99 plants were recovered (in vitro) and 15 of them were abnormal. All abnormal plants died on transfer to soilless compost but the rest were successfully established in the compost.

Purple flowers of *M. sativa* and yellow flowers of *M. falcata* are characteristic morphological feature of the two species. The presence of flowers with both purple-blue and yellow colour in 12 of these plants, indicated that they possessed

both *M.sativa* and *M. falcata* genes. The hybridity of these 12 plants was confirmed further through chromosome counts and isoenzyme analysis.

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