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# Optimisation of regeneration and maintenance of morphogenic callus in pear (*Pyrus communis* L.) by simple and double regeneration techniques

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**Abstract**

The purpose of our work was to improve the regeneration capacity of leaf explants and the maintenance of shoot morphogenesis in callus of six pear cultivars: Abate Fetel, Conference, Dar Gazi, Harrow Sweet, Kaiser and Williams, by altering the composition of both regeneration and proliferation media of explant donor shoots, and choosing the right type of explant. Regeneration capacity of leaf explants collected from in vitro shoots has been improved in the majority of cultivars also due to shoot preconditioning. For the first time, long term morphogenic callus production and maintenance have been established in some cultivars by a "double regeneration". Using this technique, morphogenic callus of two cultivars, 'Dar Gazi' and 'Conference', was maintained for several subcultures but only when they were initiated from small leaflets – less than 2–3 mm long – which had been collected from the neoformed adventitious buds. MS medium [Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Plant. 15, 473–497] proved to be an efficient regeneration medium by stimulating adventitious buds, while the explants of all cultivars, except for Kaiser, showed a high regeneration capacity when they were collected from shoots proliferated on modified QL medium [Quoirin, M, Lepoivre, P., Boxus, P., 1977. Un premier bilan de dix annees de recherche sur les cultures de meristemes et la multiplication in vitro de fruitiers ligneux. Compte rendu des recherches, Station des Cultures Fruitieres et Maraicheres de Gembloux (1976–1977), 93–117]. This medium conferred leaf expansion, overcoming 90% of regeneration in explants of cv Dar Gazi and Williams. Well expanded leaves were obtained and collected by rooting the shoots, while regeneration percentage was not improved and the number of adventitious shoots was increased in most cultivars, reaching up to 10 shoots per explant. When cefotaxime at 200 mg/l, which is normally effective in controlling *Agrobacterium*, was used for genetic transformation, regeneration percentage and number of shoots per explant (in leaf explants collected from rooted shoots) were increased and a uniform bud regeneration on all the leaf surface was promoted.


*Keywords:* Adventitious bud; Cefotaxime; Morphogenesis; $NH_4^+/NO_3^-$ ratio; Vancomycin; Double regeneration system

## 1. Introduction

The regeneration of temperate fruit trees has been reported for different explants such as stems in apple (Liu et al., 1998), protoplasts in pear and cherry (Ochatt, 1987; Ochatt et al., 1987), meristem derived callus protoplasts in apple (Saito and Suzuki, 1999), cotyledons in apples (Daigny et al., 1996) and leaves in apple and pear (James et al., 1984; Leblay et al., 1991). Among the various methods, leaves are the most important explant source used for regeneration protocols of fruit trees (Fasolo et al., 1989; Predieri et al., 1989; Abu-Qaoud et al., 1990; Leblay et al., 1991; Chevreau et al., 1997; Rugini and Muganu, 1998; Lebedev and Dolgov, 2002), and also for genetic transformation mediated by *Agrobacterium* spp. (Norelli et al., 1994; Mourgues et al., 1996; Reynoird et al., 1999).

In the last decade, several researches have been initiated to improve pear regeneration using leaf explants (Predieri et al., 1989; Abu-Qaoud et al., 1990; Leblay et al., 1991; Chevreau et al., 1997). Chevreau et al. (1997) demonstrated that the solidification of the regeneration medium by gellan gum (Phytagel™) in place of agar, positively affected adventitious bud differentiation. Leblay et al. (1991) showed that several factors, including concentrations of TDZ, $NH_4^+/NO_3^-$ ratio and initial dark exposure of the explants, significantly improved bud regeneration in pear. They suggested that among several factors, the concentration of 1 μM of TDZ, 1:3 ratio of $NH_4^+$/

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