I.5 Regeneration of Plants from Protoplasts of *Diospyros kaki* L. (Japanese Persimmon)

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1 Introduction

Japanese persimmon (*Diospyros kaki* L.) is native to East Asia and has been cultivated for centuries in China, Korea, and Japan (Tao and Sugiura 1992a). Recently, it has been gaining in popularity throughout the world and its culture is spreading to outside the temperate parts of Asia including Italy, Australia, USA, Brazil, Israel, and New Zealand. In spite of recent worldwide interest and requirements of improved fruit quality in this species, conventional cross-breeding is limited because among the hundreds of native cultivars, there are not many strains carrying hermaphrodite and/or male flowers. Furthermore, as is often the case with woody plants, the long juvenile period, large plant size, and high heterozygosity of Japanese persimmon make rapid breeding progress difficult. Therefore the use of somatic hybrids and, in particular, protoclonal variants will have a great impact on Japanese persimmon breeding programs.

To explore fully the potential of protoplast technology, an efficient and reproducible system of plant regeneration from protoplasts must be established for a given species. With Japanese persimmon, remarkable progress has been made in micropropagation through shoot tip and callus cultures during the past decade (Sugiura et al. 1986; Tao et al. 1988, Fukui et al. 1989; Tao and Sugiura 1992b). The initial report of protoplast isolation was published by Tao et al. (1991a), in which the factors influencing the efficiency of protoplast isolation were described. Later, plant regeneration from protoplasts was also achieved (Tao et al. 1991b), by using agarose-bead culture. Tamura et al. (1993) improved the regeneration efficiency by modifying the medium, and established a stable plant regeneration system for Japanese persimmon protoplasts. In this chapter, the results obtained in our laboratory concerning protoplast culture of Japanese persimmon are summarized.

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2 Isolation of Protoplasts

2.1 Source of Protoplasts

Callus culture provided good material for protoplast isolation (Tao et al. 1991a). The calli could be readily initiated in the dark from leaf primordia in dormant winter buds, on agar-solidified Murashige and Skoog's (MS) medium with half the normal strength of nitrates (1/2 N), containing 10 μM zeatin and 1 μM 3-indoleacetic acid (IAA) (Tao et al. 1988). The culture could be maintained on the same medium by subculturing at about 6-week intervals. This callus line appeared to retain its high capacity for adventitious bud formation even after 34 subcultures (Fig. 1). High frequency somaclonal variation is often troublesome when callus cells are used for protoplast isolation. With persimmon, however, there was no variation in the nuclear DNA content of the callus cells and also no morphological variation has been observed so far in the plants regenerated from the callus. Therefore, callus lines were routinely used for protoplast isolation.

2.2 Enzymes and Other Factors Influencing Yield

Age of callus, enzyme composition, and osmotic pressure of digestion mixture seem to be the key to efficient isolation of viable protoplasts. Callus growth was described by a sigmoidal curve (Fig. 2). Highest protoplast yield was obtained when calli subcultured for 1 to 2 weeks were used. This period coincided with the initial logarithmic growth phase (Fig. 2), probably leading to the high viability of the isolated cells.

Preliminary experiments showed no positive effect of hemicellulase on protoplast isolation and the best yield of protoplast was obtained when 0.5–1% Cellulase RS combined with 0.05–0.1% Macerozyme R-10 (Figs. 3, 4). As

![Graphical representation](image)

**Fig. 1.** Percentage of adventitious bud formation from callus culture of Japanese persimmon in response to culture period. *Each point* represents the data from 25 explants. (Tamura et al. 1992)