11.4 Regeneration of Plants from \textit{Linum} Protoplasts

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1 Economic Importance and Breeding Objectives in \textit{Linum}

The genus \textit{Linum} of the Linaceae family contains over 200 species, of which \textit{Linum usitatissimum} (flax) is the only one widely grown. This crop is grown either for its fibre (linen flax) or for its seed (oil flax, linseed), or for both (dual purpose flax), depending on the cultivar used and the cultural and climatic conditions. Linen thread is used in gloves, footwear, netting and sports gear. Linseed oil has many industrial purposes such as paint, varnishes, oil cloth, the manufacture of linoleum, printer's ink, soaps and patent leather, and recently as a salt-resistant coating for cement surfaces of highways and bridges.

Several of the wild species of \textit{Linum} (flax species) possess many agronomically valuable genes, including resistance to diseases such as flax rust, \textit{Melampsora lini}. These wild species are also a reservoir of genes for other important characteristics including drought resistance, winter hardiness, and an unexplored reservoir of genes for oil quality (Plessers 1966; Yermanos et al. 1966) which would be of great value if they could be transferred to flax (Seetharam 1972).

The basic fundamental problem in attempting such improvements by sexual hybridization is that crosses between wild \textit{Linum} species with nine-chromosome numbers ($2n = 18$) and \textit{Linum usitatissimum} ($2n = 30$) or any other species with 15 chromosomes ($2n = 30$) fail to produce seeds (Gill and Yermanos 1967a, b; Bari and Godward 1970; Seetharam 1972). Unfortunately, characteristics of most interest that are not found in \textit{Linum usitatissimum} are present only in some of the nine-chromosome species (Beard and Comstock 1980).

Since it has been established that somatic cell hybridization overcomes sexual incompatibility barriers to hybridization between some species (Cocking et al. 1981), it was thought realistic to try to utilize somatic hybridization of these sexually incompatible \textit{Linum} species.

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2 Source Material for Protoplast Isolation

2.1 In Vitro Seedling Material

Seeds of Linum species were surface-sterilized in 10–20% Domestos bleach for 30 min and washed in six changes of sterile tap water. Seeds were germinated on agar solidified (0.8% w/v; Sigma) Murashige and Skoog (1962) based medium (MS) containing 3% sucrose, but lacking growth regulators and incubated in the dark. Incubation was in the light for the production of in vitro-grown shoots and when green cotyledons were used for the isolation of protoplasts (2000 lx, daylight fluorescent tubes) at 23±2°C.

2.2 Cell Suspension Culture

Callus was induced on aseptic seedling cotyledon explants using agar solidified (0.8% w/v) MS medium containing 3% sucrose, 2.0 mg/l NAA and 0.5 mg/l BAP (MSP-1). Cultures were maintained at 23±2°C in continuous fluorescent light (2000 lx). Cell suspensions were initiated by transfer of 2 g fr. wt. to 50–75 ml of MSP-1 liquid medium in 250-ml Erlenmeyer flasks, with agitation on a rotary shaker (120 rpm, 2000 lx, 23±2°C). Cell suspensions were subcultured at intervals of 7 days to the same fresh medium.

3 Protoplast Isolation

3.1 Protocol for Isolation

Aseptic shoots of Linum spp. were excised after 3 weeks; young cotyledons and hypocotyls were excised after 7 days. Shoots and cotyledons were cut into approximately 1 mm-wide strips; however, hypocotyls and roots (3 days) were cut longitudinally. Tissues were plasmolyzed in CPW solution containing 13% w/v mannitol (Frearson et al. 1973) for 2 h prior to incubation in enzymes. Cells of suspension cultures 3–4 days after subculturing were allowed to settle and incubated in suitable enzyme mixture. The enzyme mixtures were E₁ 2% w/v Rhozyme HP 150 (Rohm and Hass Ltd., Philadelphia, USA), 4% w/v Meicelase (Meiji Seika Kaisha Ltd., Tokyo, Japan) and 0.03% w/v Macerozyme (Yakult Biochemicals Ltd., Nichinomiya, Japan) in CPW salts solution with 13% w/v mannitol (CPW 13M) pH 5.8; E₂ 2% w/v Rhozyme HP 150, 2% w/v Meicelase P and 0.03% w/v Macerozyme R-10 in CPW 13M and E₃ 2% w/v Cellulase R-10 (Kinki Yakult Manufacturing Co. Ltd., Japan) and 0.05% w/v Macerozyme R-10 in CPW 13M. Enzyme incubation was for 16 h at 25°C on a rotary shaker (30–40 rpm). Protoplasts were released by gentle squeezing and filtered through a nylon sieve (64 μm pore size). Etiolated cotyledon protoplasts were pelleted by centrifugation at 100 g for 10 min and washed by centrifugation using three changes of CPW