Plant protoplast technology: Current status

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Abstract

Robust and reproducible protoplast-to-plant systems are crucial for underpinning genetic manipulation technology involving somatic hybridisation and transformation. Novel and effective approaches for maximising the efficiency of such protoplast cultures include supplementation of media with surfactants and artificial gas carriers, such as perfluorochemicals and haemoglobin. Physical parameters, particularly electrosimulation, also enhance the development of protoplasts and protoplast-derived cells in culture. DNA uptake into protoplasts is now a routine and universally accepted procedure in plant biotechnology for introducing and evaluating both short-term (transient) and long-term (stable) expression of genes in cells and regenerated plants. Importantly, protoplast fusion overcomes pre- and post-zygotic sexual incompatibility barriers and generates novel germplasm through new nuclear-cytoplasmic combinations. In this respect, considerable progress has been made in generating somatic hybrid plants, particularly in citrus, brassicas and potato. Isolated protoplasts are also a unique single cell system for evaluating aspects of ultrastructure, genetics and physiology, with potential for the biosynthesis of novel secondary products, including commercially-important recombinant proteins (e.g. antibodies), and as systems in toxicity screening. Recent advances in protoplast technology have benefited from advances in animal and microbial cell culture, with interesting parallels existing between these systems. Further innovations will necessitate the strengthening of interdisciplinary links in these research fields and the requirement for continued dialogue and co-operation between workers with diverse but complementary skills.

List of abbreviations: AFLP, amplified fragment length polymorphism; CAPS, cleaved amplified polymorphic sequence; CMS, cytoplasmic male sterility; Hb, haemoglobin; HLB, hydrophilic-lipophilic balance; PEG, polyethylene glycol; PFC, perfluorocarbon; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; SOD, superoxide dismutase; SSR, simple sequence repeat

Introduction

Plant protoplast technology is not a recent concept, since important landmarks can be recognised in its development during the past 100 years. The isolation of living protoplasts, each consisting of the cell cytoplasm bounded by the plasma membrane and free from the normal constraints of their primary enveloping walls, was initiated by the pioneering work of Klercker (1892) who, after slicing leaves of the water plant, *Stratiotes aloides*, extruded the...
living cell contents into a hypertonic solution. Several years later, what may now be considered as early ‘biotechnology’ experiments emanated from investigations of Kuster (1909) and Michel (1937). These workers demonstrated that the plasma membranes of isolated protoplasts (wall-less ‘naked cells’) could be induced to fuse, permitting coalescence of their cytoplasms. However, such investigations passed virtually unnoticed for many years until the commercial availability, during the 1960s, of wall degrading pectinases and cellulases. Such enzymes, when used either sequentially or in combination, facilitated the reproducible isolation of large, workable populations (>1 x 10⁶) of viable protoplasts from primary tissues of a range of plant species.

When cultured in the laboratory, isolated protoplasts and their derived cells are unique in that, theoretically, they are totipotent, each being able to develop into one or more fertile plants. Studies focused upon protoplast isolation and culture initially resulted in the regeneration of fertile plants from protoplast-derived cells of tobacco (Nagata and Takebe 1971), with extension to a range of other species during the 1970s and 1980s. Such protoplast-to-plant systems permitted the initiation of genetic manipulation experiments involving somatic hybridisation/cybridisation by protoplast fusion, and transformation by uptake of DNA. The 1990s witnessed the introduction of novel approaches to increase the efficiency of protoplast culture and plant regeneration through electrostimulation, and the incorporation of surfactants and artificial oxygen carriers into media (Lowe et al. 2003a). Such work resulted, primarily, through technology transfer from animal and microbial cell culture systems. Advances in cell culture, accompanied by improvement in chemical fusion, electrosfusion and DNA uptake procedures, have facilitated the generation of plants expressing new gene combinations (Davey et al. 2000a). Indeed, it is noteworthy that there is a resurgence of interest in somatic hybridisation/cybridisation to circumvent naturally occurring sexual incompatibility barriers and to generate genetically novel germplasms for breeders. Whilst the emphasis in terms of DNA uptake has been on nuclear transformation, and will continue in this area, the introduction of genes into organelles (plastids) is gaining momentum (Maliga 2003). In addition to their exploitation in genetic manipulation, isolated protoplasts feature as experimental material in a range of anatomical, physiological and genetical studies that are reviewed in this article.

Isolation and culture of protoplasts

Whilst the majority of studies have focused on the enzymatic isolation of protoplasts of higher plants, there are also examples of the preparation of osmotically-fragile protoplasts from mosses (e.g. Physcomitrella patens; Hohe and Reski 2002) and algae (e.g. Bryopsis plumosa; Yamagishi et al. 2004). Interestingly, the release of protoplasts from Bryopsis involved a mechanical procedure by cutting of gametophyte or sporophyte tissues with sharp scissors. Currently, large populations of protoplasts can be isolated from tissues of expanded leaves and petals, seedling organs (including hypocotyls, cotyledons and roots) and dedifferentiated or embryogenic cell suspensions, the latter being the preferred source material for monocotyledons, such as cereals and banana (Assani et al. 2002). The advantage of seedlings is the short period, usually only a few days, from sowing of the seeds to excision of explants for protoplast isolation. Protoplasts have also been isolated from highly specialised tissues, such as guard cells of Arabidopsis thaliana (Pandey et al. 2002), these cells being excellent source material for totipotent protoplasts. Gummadi and Panda (2003) and Doi and Tamaru (2001) have discussed the use of cell wall degrading enzymes for protoplast isolation, concentrating upon microbial pectinases.

Under the appropriate cultural conditions, isolated higher plant protoplasts, which are normally approximately 20-50 µm in diameter, synthesise new cell walls and, more or less synchronously with this, enter sustained mitotic division. Daughter cells develop into tissues from which plants may be regenerated by somatic embryogenesis and/or organogenesis. Several procedures have been described to culture isolated protoplasts, with incubation in liquid medium being the most simple to establish. Protoplasts have also been cultured in media overlaying supports of nylon mesh and filter pa-