related or even different organisms. The application of plant biotechnology to complement traditional hybridisation offers the opportunity to meet such challenges.

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Latest Developments in Plant Breeding: Application of Biotechnology©

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INTRODUCTION

The generation of new plant varieties is an on-going process that provides material for the commercial market. Conventional breeding involving self and cross-pollinations, fertilisation, and seed production followed by rigorous selection of plants with desirable traits, is a long-established and reliable procedure for generating novel germplasm. In addition to this traditional approach, techniques involving the culture of plant cells, tissues, and organs in the laboratory provide a means for direct and indirect plant improvement. A unique characteristic of plant cells is their so-called totipotency—individual viable cells of almost any origin carry the genetic information needed to develop into complete fertile plants. This developmental pathway can be induced in the laboratory. Major advances have been made in plant biotechnology during the last two decades, several of which exploit cell totipotency, the basis of plant tissue culture, and which can underpin conventional breeding and propagation.

Thus, tissue culture-based procedures are being used to mass propagate and generate elite plants, to create new hybrids between sexually incompatible genera and species and to introduce specific traits into target plants using recombinant DNA-transformation technology. The target for genetic modification is DNA (deoxyribose nucleic acid), with its base sequences characteristic of the species, located in the nucleus, plastids and mitochondria of cells. DNA is the material of which genes are composed. Biotechnological approaches based on plant cell totipotency generate novel germplasms of interest to propagators, breeders, and seed merchants.

CULTURE OF CELLS, TISSUES, AND ORGANS IN THE LABORATORY

Procedures for introducing and establishing material in culture have been summarised by Hall (2000), who provides excellent advice relating to laboratory conditions, choice of media, and equipment to facilitate the tissue culture process. Shoots and rooted plants can be readily maintained in culture, while explants (portions of leaves, stem, roots, and floral organs) can be stimulated to undergo adventitious (direct) shoot regeneration. Additionally, explants will produce callus, a mass of loosely connected cells which can be maintained in an undifferentiated condition, or be induced to regenerate shoots by organogenesis and/or somatic embryogenesis. The latter two processes are regulated, primarily, by the choice of cells. Callus will dissociate in agitated liquid culture medium to produce actively growing cell suspensions from which plants can be regenerated, again from individual cells. Totipotent protoplasts (wall-less cells) can be isolated from leaves, hypocotyls, stems, roots, petals, and cell suspensions and, for many plant species, used for making new (somatic) hybrids by fusion.

MICROPROPAGATION: MASS PRODUCTION OF PLANTS AND SECURING OF "SPORTS" (NOVEL PLANT FORMS)

The culture of explants on a suitable medium usually containing cytokinins at an elevated concentration relative to auxins, results in direct shoot differentiation at the cut surfaces of the explants. Thus, from a parental plant, it is possible to generate hundreds of new individuals from one or more detached leaves, at the same time retaining the original plant. For example, in African violet, 1 sq cm leaf explants each produce several shoots, enabling up to 5000 plants to be regenerated from a single expanded leaf. In contrast, only between 1 and 5 shoots usually develop from traditional leaf cuttings. Likewise, each petal of individual flowers of African Violet will regenerate about 500 plants.

In chrysanthemums, new floral colours and patterns often arise by "sporting" which may involve the whole inflorescence, or it may be limited to a few florets or to parts of one or more petals. Inflorescence sports may be secured by vegetative propagation of the pedicel, provided the genetic change (mutation) which produced the sport extends down the pedicel. However, sports limited to a few florets, or to parts of the petals of a floret, cannot be secured in this way because of the limited extent of the mutation. Nevertheless, plants can be regenerated readily directly from sporting petals when the latter are excised from the parent flowers and introduced into culture (Malaure et al., 1991). Petal sports have been secured in the spray cultivar chrysanthemum 'Enbee Wedding' with registration of the new cultivar 'Pearl Enbee Wedding' by the Royal Horticultural Society in 1994 and plants subsequently being offered for sale by Woolmans of Dorridge, UK.

While the majority of plants regenerated from explants or from explant-derived callus will be true to parental type, variants can often be discovered following large-scale glasshouse and field screening of regenerated plants. For example, in a trial of lettuce plants derived from leaf callus some were found to be dwarfed with dissected leaves (Brown et al., 1986). Somaclonal variation may be stable through seed generations, providing useful material for incorporation into conventional breeding programmes (Karp, 1995). However, the origin of somaclonal variation remains unclear. It may be attributed to natural changes in the genetic composition of the original cells, the expression of which is facilitated in regenerated plants by the culture process.

ISOLATION AND CULTURE OF PLANT PROTOPLASTS

Protoplasts are live plant cells which have been treated with digestive enzymes which remove their cell walls to leave the thin cell membrane exposed. These have become a valuable tool in hybridisation as they offer a means to bring together the genetic material of otherwise incompatible species. Source materials for protoplast isolation include seedling cotyledons, roots and hypocotyls, leaves from cultured shoots or glasshouse-grown plants and cell suspensions. Even specialised cells, such as root hairs, pollen grains, and tetrads will release protoplasts with the potential to undergo division and differentiation to produce whole plants.

Isolated protoplasts can be cultured in several ways (Davey et al., 2000a) and their cell walls will regenerate soon after removal from the enzyme solution, with protoplast-derived cells entering sustained mitotic division usually within 24 to 72 h. Tissues derived from totipotent protoplasts will each regenerate one or more shoots through organogenesis or somatic embryogenesis, depending on species and/or mode of culture. Some regenerated plants may exhibit elevated levels of somaclonal variation, as discussed earlier, due to the longer time-frame required for regeneration.

EXPLOITATION OF THE TOTIPOTENCY OF ISOLATED PROTOPLASTS: SOMATIC HYBRIDISATION AND CYBRIDISATION

Breeders frequently encounter naturally occurring pre- or post-zygotic incompatibility barriers, based on complex physiological and/or genetic mechanisms, which prevent seed development. In some cases, pre-zygotic incompatibility may be overcome by culturing excised flower buds, followed by their hand pollination. Post-zygotic incompatibility can be circumvented by isolating and culturing developing hybrid embryos (embryo rescue).

Alternatively, somatic hybridisation, involving the chemical or electrofusion of isolated protoplasts (Davey et al., 2000b), can be used to combine the entire genomes of two species, even of different genera. Both homokaryons and heterokaryons are generated, since fusion is a random process. Heterokaryons are the most important in terms of genetic novelty. Initially, they consist of the nuclear genomes of both parents, together with their plastids and mitochondria in a mixed cytoplasm. Subsequent developmental stages are complex. Hybrid cells may result which retain the nuclear genetic materials from both parents (complete hybrids) or which may lose chromosomes from one or both parents (partial and asymmetric hybrids).

A diverse array of phenotypes may emerge, depending on which chromosomes are retained. The plastids from one parent usually come to dominate, with those of the other parent being lost. In contrast, the respective mitochondria usually undergo

DNA recombination. Cybrids result when the nuclear genome of only one parent is retained in a mixed cytoplasm with the plastids from the other parent and/or recombined mitochondria. Irradiation of protoplasts of one partner before fusion will fragment nuclear DNA to promote cybridisation under a positive selection pressure, thus mediating transfer of cytoplasmically-encoded characteristics. The selection of somatic hybrid tissues with desirable characteristics, prior to plant regeneration, remains the most difficult part of somatic hybridisation (Blackhall et al., 2000, Andras et al., 2000).

Somatic hybrids have been reported in several plant families, particularly genera and species within the Solanaceae, Brassicaceae, and Compositae. The technology is now being extended to monocots, including ornamentals and cereals. Somatic hybridisation enables large amounts of genetic material to be combined without the need to know its location or to isolate DNA in the laboratory. Cybrids enable cytoplasmically encoded traits, such as plastid-encoded herbicide resistance and mitochondrial-encoded male sterility, to be mobilised between plants. Overall, the genetic composition of plants generated by protoplast fusion is usually more complex than that of plants produced by conventional breeding. This generates even greater genetic novelty for the breeder.

RECOMBINANT DNA TECHNOLOGY AND PLANT TRANSFORMATION

Recombinant DNA procedures, combined with transformation, provide a range of alternative "high technology" approaches for genetically manipulating plants, although they are still reliant, in most cases, on the tissue culture process. Genes for transfer can be isolated from micro-organisms, plants, and animals. The DNA can be cut at specific base sequences with restriction enzymes, modified, and rejoined using enzymes (ligases). Thus, chimaeric genes constructed in the laboratory can be introduced into plants using various delivery approaches (Newell, 2000), with those in routine use based on the natural gene transfer mechanism of the soil bacterium *Agrobacterium tumefaciens* (Dumas et al., 2001) following inoculation of explants which readily regenerate shoots (e.g., leaf discs). Biolistic procedures involve the coating of particles (usually gold because of its inertness) with DNA and their introduction at high speed into totipotent tissues using devices such as the Bio-Rad PDS-1000/He helium driven instrument. Frequently, this approach has been employed to transform plants which do not respond readily to *Agrobacterium*-mediated gene delivery; the mechanism of release of DNA from the gold particles and its integration into the recipient plant genome is not understood.

The selection, after gene delivery, of transformed tissues and, subsequently, transgenic shoots, is usually based upon resistance to antibiotics or herbicides, the genes for which are introduced simultaneously with the gene(s) of interest.

Target plants for genetic modification include most cereals, oilseeds, fruit, fibre, pulse and tuber species, woody species, and ornamentals. Since most crop losses result from attack by viruses, fungi, bacteria, insects, and invasive weeds, it is not unexpected that increased resistances to these pests have been the main targets for genetic manipulation using recombinant DNA-transformation technology. Other targets though include modification of plants for the safe and efficient biosynthesis of novel pharmaceutical products, altering flower colour, improving nutritional quality, dwarfing to reduce the use of potentially unsafe chemical growth retar-

dants, regulating fruit ripening and delaying senescence, the latter in, for example, leafy vegetables.

Clearly, the ability to introduce one or more genes into plants, enabling rapid modification of specific trait(s), is a major outcome of recombinant DNA-transformation approaches compared to mass gene transfer through protoplast fusion or conventional breeding. The main difficulty, at present, in fully exploiting the recombinant DNA-transformation approach, unlike somatic hybridisation, is in the reluctance of consumers, and possibly breeders, to accept plants generated by this technology, since their genetic manipulation raises important environmental considerations, ethical issues, and public concerns.

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