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Plant cell biotechnology and agriculture: impacts and perspectives

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Methods of cell and tissue culture, together with the regeneration of whole plants, are now routine for many plant species and are rapidly becoming key tools in the further development of agriculture and horticulture. At the base of the technology lies the ability to produce large numbers of identical (cloned) plants far more rapidly than with traditional practice. A number of commercially important species, including strawberries, tomatoes, oil palms, Douglas firs and orchids are already being produced in large numbers through tissue culture.

A further dimension is provided by techniques of protoplast-fusion–somatic-hybridization and genetic manipulation. Harnessed to methods of automated mass propagation it should be possible to screen for, and develop much more swiftly, new commercially important varieties than was possible hitherto with conventional plant breeding approaches.

In spite of good progress in many aspects of plant tissue culture there is, however, much to be learned about the nature of the system. Techniques of plant tissue culture are by no means yet generally applicable.

Introduction

In recent years much progress has been made in plant cell biotechnology, particularly in those areas concerned with the propagation of plants and the development of new plant varieties. It is with this aspect of plant cell biotechnology that this short review is concerned.

The vegetative propagation of plants in horticulture and agriculture together with associated regenerative phenomena is well established. Over the years a wide range of vegetative propagation techniques have been developed and used with varying degrees of success. The advent of cell, tissue and organ cultures and the regeneration of plants through such systems has added a further dimension to traditional technology. Micropropagation, as tissue culture is more usually called today, has a number of key attributes additional to those seen for traditional or conventional propagation techniques. It is eminently suited to rapid multiplication of clonally derived material, it can be used to derive and multiply disease-free stock, it may allow the multiplication of sexually derived non-fertile crosses (this would allow, for instance, the use of F1 hybrid material in its own right), and through protoplast fusion and formation of somatic hybrids may provide a further approach to developing new hybrids with desirable characteristics or traits. Unfortunately the ease with which the above is stated belies the difficulties often encountered in developing a micropropagation route for any particular species or variety. While there is undoubtedly great potential in micropropagation, and a number of species are being commercially propagated in this way, there is much to learn about the system before full scientific, technical and commercial success is achieved. It is the aim of this short review to assess developments in micropropagation in terms of that which is desirable against that which is currently feasible.

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Plant propagation through tissue culture

A wide range of systems have now been developed for the production of plants through tissue culture and these are summarized in figure 1. Space does not allow a detailed discussion of these systems; instead, the salient features will be highlighted.

Murashige (1974) delineated three stages in the micropropagation process; (i) the selection of suitable explant material, (ii) the proliferation of shoots on a multiplication medium and (iii) the transfer of shoots to a rooting medium, followed by planting out. While these headings are broad-ranging they nonetheless provide a useful breakdown of the key steps through which a micropropagation programme has to proceed.

Of crucial importance is the origin and nature of the explant material used to initiate the propagation process. In the main, micropropagation typically involves regeneration through shoots and shoot primordia (meristems) (figure 1). *In vitro* cultures may then be initiated from the embryo, main apex or axillary buds. It is also possible to initiate cultures from tissue or organ explants. In this case morphogenesis may be induced directly through adventitious shoots or somatic embryos, or indirectly through callus or suspension cultures.

Whatever the chosen route for propagation it is essential that the starting material comes from clean, healthy plants. Higher plants possess an extensive surface microflora and internal infection is also not unknown. In consequence and to prevent microbial spores passing through into culture with all the resultant problems, intensive efforts are used to render sterile the outer surface of the explant. This typically involves sterilizing the explant with agents such as hypochlorite, mercuric chloride or ethanol.

A key aspect of the propagation of plants through tissue culture is the development of an appropriate nutrient formulation upon which the cultures are grown and which induces shoot, embryo or root formation. The nutrient formulations for tissue cultures have five major components: macronutrients, typically the salts of the common acids; micronutrients; a nitrogen source; a carbon source and plant growth regulators. The formulation of the nutrient medium has to be carefully adjusted to provide conditions that favour culture initiation, development and plant regeneration. Each of these stages may require a slightly different formulation, the development of which can often be a painstaking and tedious process. There are, however, a number of 'standard' formulations that have been shown to induce shoot formation in a variety of systems. These typically rely on 'appropriate' relative concentrations of plant growth regulators to induce desired effects. Perhaps the best known situation is that described by Skoog & Miller (1957) where, in a range of species, a relatively high cytokinin to auxin ratio leads to shoot formation. It should be noted that the absolute levels of plant growth regulators required may vary in each case.

In addition to nutrient supply, light and temperature also play important roles in the establishment and further development of explants. Although few tissue systems exhibit any sign of photosynthetic competence, light appears to be necessary for regeneration. Most species are grown under light intensities of 1000–5000 lx with daylight fluorescent tubes. Murashige (1977) has advocated the use of 16 h days to enhance regeneration phenomena, but there has been comparatively little experimentation or discussion about this. A similar picture is true for temperature, where most plants are grown in the range 21–25 °C, although there are reports of plants with optima as far apart as 18 and 30 °C (Hussey 1983).

Once a decision has been taken as to which part of the mother plant should be used for explant

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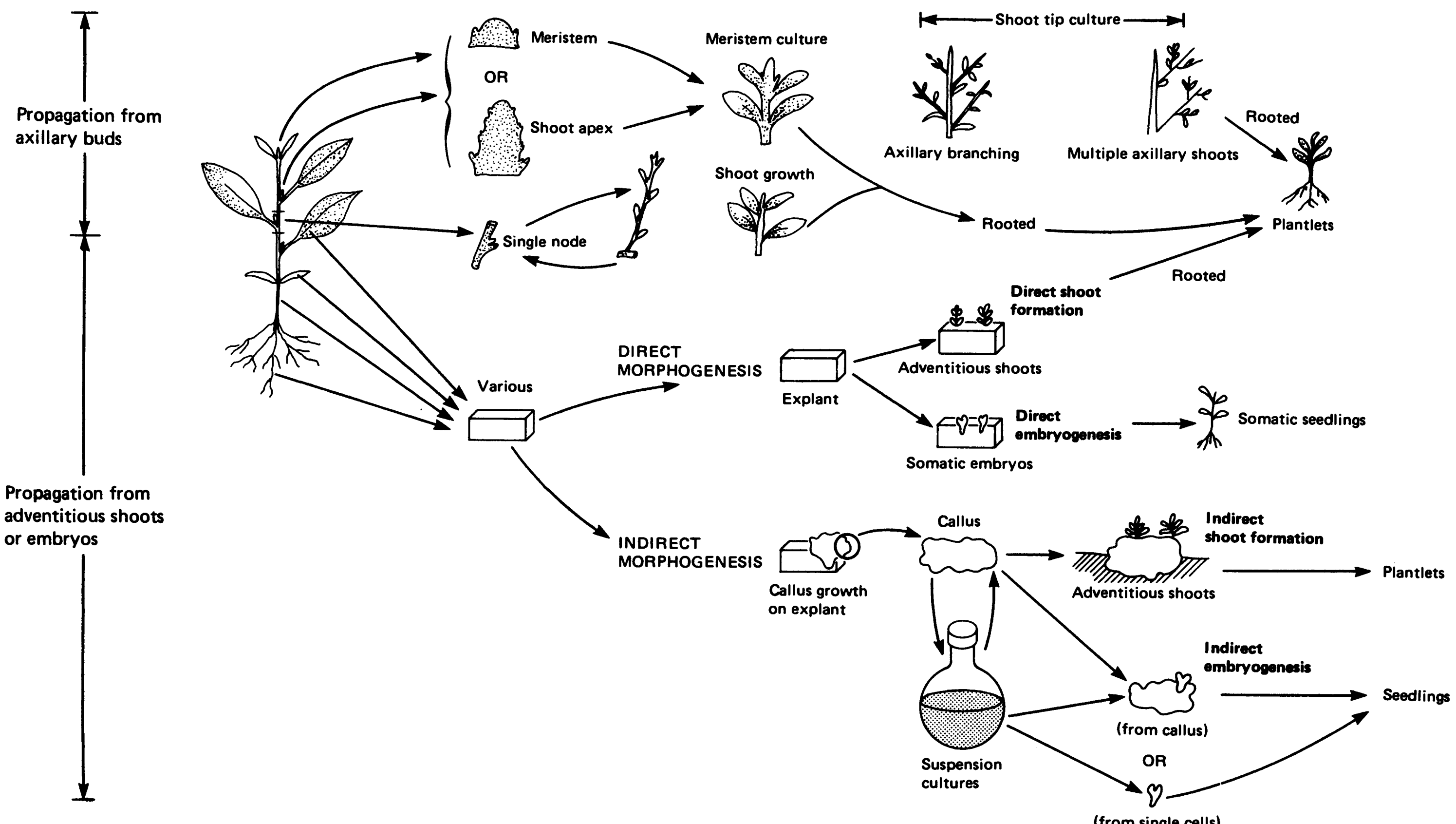

Figure 1. The principal methods of micropropagation. Reproduced with permission from E. F. George & P. D. Sterrington, *Plant propagation by tissue culture* (Exegetics Ltd, 1984).

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purposes, the path ahead is fairly straightforward. In the case of the axillary buds the explant is placed on an agar-based nutrient containing high cytokinin levels. Quite often these apices begin to produce secondary, tertiary and higher shoots in a cluster. In time this cluster may be divided and the individual pieces put onto fresh nutrient media. The individual pieces will form new clusters from which shoot structures may be removed and so on. The rate at which shoots can be proliferated in this way varies from case to case but can be as high as 5–10 plantlets per explant over a few weeks. This approach is rapidly becoming a standard one in micropropagation.

An alternative approach is through adventitious embryogenesis (figure 1). Here, explants are taken from various parts of the plant and induced into either direct or indirect embryogenesis. Through direct embryogenesis shoots or somatic embryos may be formed directly on the explant. When shoots are formed the problem is as described previously, i.e. inducing root formation at the base of the shoots. For somatic embryo formation, a balanced system of shoot pole and root pole develop and eventually a seedling plant is obtained. Indirect embryogenesis first involves the formation of a callus, a non-differentiated mass of cells on the surface of the explant. The callus can then be dealt with in two ways, either it may be induced to form somatic embryos on the surface as above, which in turn gives rise to balanced plantlets, or it may be placed in liquid culture. In liquid culture the callus mass is gently agitated and cells are released. These cells grow and divide and, given the appropriate environmental conditions, will become embryogenic. In time these tiny embryos develop into seedlings and then plants. It is this latter route that may provide the greatest promise for the use of micropropagation in agriculture for mass food crops, a point to which I will return later.

Plants produced through tissue culture

A wide range of plants have now been regenerated through plant cell, tissue or organ culture. Some of the better known examples are listed in table 1. In table 2 are listed reasons for the use of micropropagation in relation to some species, as perceived by the Congress Office of Technology Assessment in the U.S.A.

Of the plants propagated commercially through micropropagation most, as mentioned above, are regenerated through shoot tip culture. This approach is both labour-intensive and

Table 1. Examples of plants propagated through tissue culture

horticulture	agriculture: speciality crops
African violet	oil palm
begonia	citrus
chrysanthemum	date palm
dracaena	jojoba
gerbera	
gloxinia	
rose	
rubber plant	
orchid	
sylviculture	**agriculture: mass food crops**
Douglas fir	beet
loblolly pine	brussel sprouts
redwood	cauliflower
	lettuce
	spinach
	sweet potato

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TABLE 2. ECONOMIC BENEFITS AS PERCEIVED FOR CERTAIN U.S. CROPS[a]

industry	application	economic benefit
asparagus	rapid multiplication	improved productivity and quality
citrus	virus elimination	improved quality, high productivity
coffee	disease resistance breeding	healthier stock
pineapple	mass propagation	higher productivity, improved quality
strawberry	mass propagation	rapid introduction of new strains

[a] Modified from Office of Technology Assessment, U.S.A. Congress, Impacts of Applied Genetics, p. 143, 1981.

costly, and can only really be justified in the case of high-value speciality plants, as for instance with horticultural species, speciality agricultural crops, or sylviculture. A particularly good example of this situation is seen with the oil palm. Unilever have developed a micropropagation system based on labour-intensive techniques, but the nature and value of the micropropagated plant is such as to make the approach worthwhile. In this case, embryogenic oil-palm cultures have been developed to provide clones of desirable, uniform and consistent characteristics (Jones 1983*a*, *b*), all of which allows sale at a high added value. Such a situation will only occur with the mass food crops if a major development in technology occurs. It is generally considered that this will have to be in the area of automated propagation and somatic embryogenesis. One possible approach would be to induce callus or explants from the target species and then to place the callus in liquid suspension culture. Once established in liquid as a cell suspension, an appropriate signal, either chemical or physical, could be administered to initiate embryogenesis but in a synchronous fashion. The next step would require an automated handling system to select discretely either embryogenic centres or embryos before organ development became too advanced, in turn leading to problems of separating individual plantlets or seedlings from a tangled mass of leaf primordia, shoots and roots. Once separated the individual embryogenic centres could then be developed on to plantlet stage, potted on, hardened off and then planted out in the field, possibly through some form of fluid drilling.

While such a scenario has considerable appeal and while there is much from a commercial and agricultural practice standpoint to commend it, it must be said that we are far from achieving such a goal. Major scientific and technical hurdles have yet to be overcome. First, only in a very few species has somatic embryogenesis in liquid culture yet been achieved (Hussey 1983). Second, there are indications that populations of plants derived through this route may not be as uniform in characteristics as desired, compared, for instance, with those propagated through shoot tip culture. Third, while some success has been achieved in synchronizing somatic embryogenesis in some species, little is understood of the precise biochemical and physiological mechanisms that 'signal' the onset of embryogenesis. (It is probably in this area that the greatest problems for research and development will occur.) Fourth, the whole area of mechanical handling from the embryo culture vessel down to the field has yet to be fully addressed. This latter will involve close collaboration between plant scientists, and control and mechanical engineers. It is possible that presently developing techniques of microencapsulation may play a key role in automated handling. Developments in the United States and Europe point in this direction.

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New varieties

So far we have been concerned principally with 'more of the same'. Professor P. R. Day, in his contribution to this symposium, has detailed some of the key developments presently occurring in breeding and genetic engineering in crop plants. Micropropagation could play, and in some cases is already playing, a major role in this. The ability to produce large numbers of plants at high levels of uniformity from shoot-tip culture in particular allows the breeder to reach the field trials stage much earlier than by traditional techniques. In some cases this may shorten the breeding cycle by as much as 50%.

Regeneration of plants following the fusion of protoplasts from different varieties may also be of major importance in the future (Cocking 1983). Such an approach allows the rapid proliferation of large numbers of plants with which to screen for and consequently develop clones and populations having altered, desired characteristics.

Perspective

Quite obviously in a review of this length it is only possible to cover superficially what is a rapidly developing area, and to highlight key points. As a general comment it is undoubtedly true that micropropagation and associated aspects, such as protoplast fusion, have much to offer plant agriculture in the future. Furthermore, good progress has been made in many aspects of micropropagation. At the moment, however, much of the commercial activity is coupled to horticulture and the speciality crops where the high costs associated with micropropagation are covered by the high added value of the product itself. Major technical developments need to take place, particularly in automated propagation, before mass food crops will come into the picture. It might be argued that micropropagation will contribute more to plant breeding and the development of new varieties in the immediate years ahead, than it will to mass propagation of general crops out in the field. However this should not be allowed to reduce efforts to develop the latter. The potential rewards for agriculture and the well-being of mankind are too great for it to be cast aside.

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