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Potato Protoplasts and Tissue Culture in Crop Improvement

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Introduction

Potato (*Solanum tuberosum* L.) is one of the most important and widely grown food crops in the world. The cultivated forms originate from a narrow genetic base but 160 wild species are recognized and the global gene pool is relatively untapped (Hawkes, 1978). Commercial cultivars are tetraploid ($2n = 4x = 48$) and extremely heterozygous, with simplex inheritance (Aaaa) for many characters (Howard, 1978). Some do not flower easily, or have reduced fertility, and others are pollen sterile, thereby limiting the number of desired crosses that can be made (Howard, 1970). Cytological studies have helped towards understanding the relationships of different *Solanum* species, but the chromosomes are small and difficult to distinguish. Such knowledge as the chromosomal localization of important genes, which has aided cereal breeding, is therefore largely not available for potato.

Most new potato varieties are made by crossing together parents with useful characters followed by vegetative propagation of the F_1 plants to form clones. These clones and their tuber progenies are then screened in gradually increasing plots over several years for favourable combinations of agronomic traits. Effort has also been focusing on the use of true seed for breeding programmes.

Over the last 15 years a new approach to potato breeding has been emerging. At the outset, this approach should be viewed as a complement to conventional breeding practices, and not as a replacement. There have been technological advances in the developing fields of plant cell and molecular biology. Tissue culture systems provide the experimental system to which

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Abbreviations: AEC, 5-(2-aminoethyl)cysteine; BAP, Benzylaminopurine; DDMH, doubled double monohaploid; DMH, double monohaploid; DNA, deoxyribonucleic acid; 5MT, 5-methyltryptophan; NAA, naphthalene acetic acid; PEG, polyethylene glycol; PLRV, potato leaf roll virus; PVY, potato virus Y.

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techniques of genetic manipulation can be applied. Potato is one of the more amenable crops for tissue culture. Whole plants can be regenerated from a range of cultured tissues (roots, leaves, tubers, stems) and from single cells. This technology offers new potential for potato breeding in a number of ways: some tissue culture techniques can be incorporated into breeding programmes to improve efficiency; new breeding strategies can be designed using tissue culture in conjunction with conventional breeding methods; new sources of variability are available, and a new type of genetics can be applied by coupling the regeneration systems with the advancing technology of genetic engineering.

In this review we briefly describe the tissue culture techniques that can be applied to potato, and the way in which some can be utilized in breeding programmes. We then examine some of the consequences of the culture techniques and the new source of variation that has unexpectedly arisen in certain tissue culture systems. We then review the progress that has been made in the developing areas aimed at more direct manipulation of the genome. Our emphasis is placed not so much on the techniques themselves as towards an assessment of their potential for crop improvement.

Tissue culture responses and their application

Potato is amenable to a number of tissue culture techniques, ranging from *in vitro* propagation via shoot cultures to regeneration of whole plants from protoplasts. In general terms, these all involve the growth of plants, cells, tissues and organs in sterile conditions, supported by an appropriate culture medium. Media normally contain a mixture of major and minor salts, vitamins, sugar (as a carbon source) and plant growth regulators. The most widely used formulations are based on that of Murashige and Skoog (1962), which is available commercially.

VIRUS ELIMINATION BY MERISTEM-TIP CULTURE

Potato is susceptible to many viral pathogens, some of which may be present without obvious symptoms, although causing gradual decrease in vigour and yield. It is therefore very important, in this vegetatively propagated crop, to be able to eliminate viruses and obtain disease-free plants.

Eradication of viruses can be achieved by culturing excised meristematic buds under appropriate conditions, a method that has been successfully applied in many countries. The techniques are described by Mellor and Stace-Smith (1977) who list 136 virus-free potato cultivars produced by meristem-tip culture. The general procedure involves pretreatment of potato shoots by growth at raised temperatures (32–37°C), surface sterilization and excision of apical and axillary meristems (length 0·3–0·7 mm) and transfer of the excised meristem to a filter paper bridge in a tube containing liquid culture medium. After culture at 20–25°C, shoots emerge and can be rooted and potted out. The treated plants are then tested for the presence of viruses.

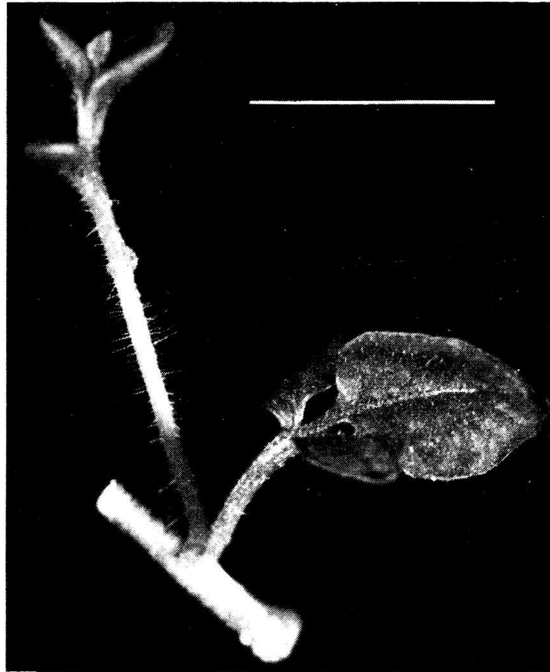

Figure 1. A shoot culture: development of a shoot from an axillary bud on a cut stem (Scale bar = 1 cm).

Critical factors are the size of the meristem and heat treatment. The addition of antimetabolites may also assist virus eradication.

MICROPROPAGATION AND GERMPLASM STORAGE

Axillary buds on excised stem segments in culture will grow out to form shoots (*Figure 1*). Shoot cultures of potato obtained in this way can be maintained indefinitely by repeated subculturing. They are a valuable source material for tissue culture and a means of keeping genetic stocks. They also provide the basis for rapid multiplication of potato stocks by micropropagation.

Following surface sterilization, stem segments are cultured on standard agar media, in the absence of growth regulators, or with low levels of cytokinin (Hussey and Stacey, 1981). Shoots which develop can, in turn, be cut into nodal segments to repeat the process. A multiplication rate of about × 10 per month occurs under continuous light (6000–8000 lux) at 25°C and, by 18 weeks, over 500 plants can be obtained from sprouts from one medium-sized tuber (Hussey and Stacey, 1981). Cultured shoots rapidly develop roots and can be transferred to soil after washing off the agar. Alternatively, stem segments can be cultured, rooted in liquid medium and transferred directly to soil, a method which is more convenient but which results in lower final multiplication rates (Hussey and Stacey, 1981).

After 3–4 months in culture, mini-tubers of 3–6 mm diameter may develop at the nodes. Production of one per node can be obtained effectively by culturing stems in short days on medium containing high benzylaminopurine (BAP) and sucrose levels (2·0 mg/l BAP, 6% sucrose). Mini-tubers exhibit dormancy and may be used for storage and transport of germplasm (Hussey and Stacey, 1984).

Micropropagation is a useful means of multiplying virus-free potato stocks and new or imported cultivars, for which few tubers may be initially available, under conditions where reinfection with virus or infection with fungus-borne diseases does not occur. It is used commercially, for example by Nickersons (Scotland) as a means of multiplying first-year stock for seed production. The propagation of virus-tested stem cuttings by conventional methods produces 800–900 plants from a single clone in 3 years (Hussey and Stacey, 1981). In contrast, by using *in vitro* micropropagation many thousands of plants can be produced from one clone in a single year, although at least one generation in the field is required before use.

In order to maintain genetic resources in potato, there is a need to store primitive potato cultivars and related wild species which cannot be stored as seed. In conventional propagation there is always a risk of loss (Westcott, Henshaw and Roca, 1977). Clones may be stored by the techniques outlined above, but using additional modifications to slow the growth in culture and to lessen the need for subculturing. This can be usefully achieved by growth at low temperature (e.g. 6–8°C) and low light intensity, which requires only annual subculturing. In future the need to subculture may be eliminated completely by cryopreservation in liquid nitrogen (Withers, 1983).

ORGAN AND EXPLANT CULTURE

Whole potato plants can be regenerated from cultured organs, such as anthers and ovaries, and from cultured explants, such as pieces of leaf, stem, rachis and tuber. Regeneration procedures generally involve the production of adventitious shoots from disorganized cell growth, or callus, unlike meristem-tip culture and micropropagation, in which callus formation is avoided.

Regeneration from cultured explants has been achieved by use of a single medium for both callus initiation and shoot formation (Roest and Bokelmann, 1976; Jarret, Hasegawa and Erickson, 1980). A simple two-stage procedure has been applied to a broad range of cultivars (Webb, Osifo and Henshaw, 1983; Wheeler *et al.*, 1985) for monohaploid and dihaploid lines (Karp *et al.*, 1984). The cell proliferation phase, of about 2 weeks' duration, requires the presence of auxin and cytokinin in the medium. In a second morphogenetic phase, adventitious shoots appear on a medium containing cytokinin and gibberellic acid (Webb, Osifo and Henshaw, 1983; Wheeler *et al.*, 1985). Callus formation occurs at the cut surfaces of explants by the 10–14th day. The callus becomes nodular with the emergence of shoots after 24 days and large numbers of shoots (more than 50) may form on 1 cm leaf discs or rachis pieces (*Figure 2*). The shoots can be rooted by transfer to medium containing 0·06 mg/l naphthalene acetic acid (NAA) and then transplanted into soil to produce full-size potato plants.

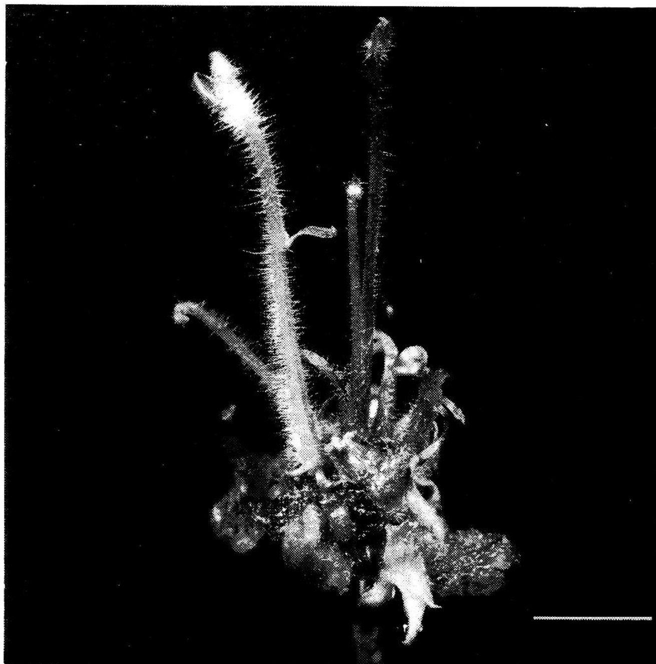

Figure 2. Shoots regenerated from callus formed on cultured leaves of a monohaploid potato (Scale bar = 1 cm).

Anther and ovary culture for the production of haploids

Anther and ovary culture can be used as a means of producing potato plants with a gametic chromosome constitution (or haploid potatoes). As potato is a tetraploid, two successive levels of haploidy are possible: the first, or dihaploid ($2n = 2x = 24$) is obtained after reduction from the tetraploid; the second, or monohaploid ($2n = x = 12$) after reduction from the dihaploid. Both levels are useful in potato breeding (Hermsen and Ramanna, 1981).

In addition to anther and ovary culture, haploids can be obtained by parthenogenesis or chromosome elimination and can also arise spontaneously. To be of value, tissue culture should therefore be a more efficient alternative. Parthenogenetic extraction of dihaploids by crossing tetraploid potatoes with *Solanum phureja* (Hougas and Peloquin, 1957) has been so improved by the use of 'superior pollinators' that production by this method is relatively routine and has superseded anther culture. Recent studies by Johansson (1986), however, have shown a much improved efficiency of dihaploid production in anther culture of several potato cultivars. Out of 20 tetraploid clones, 19 produced embryoids and more than 90% of the regenerated plants were dihaploid. These studies indicate that tissue culture may still make a contribution to dihaploid production.

Parthenogenetic extraction has been used to obtain monohaploids from dihaploids (Jacobsen, 1978), but efficiency is low and advances in the culture of dihaploid anthers have led this to be the favoured technique for certain

genotypes (Binding *et al.*, 1978; Jacobsen and Sopory, 1978; Sopory, Jacobsen and Wenzel, 1978; Wenzel *et al.*, 1979).

The factors that are important for anther culture include background genotype, plating at the correct developmental stage (uninucleate microspores, i.e. flower buds 4–6 mm in length), pretreatment (e.g. incubation of flower buds at 6°C for 48 h) and the media components (e.g. sucrose 6%, activated charcoal 0·5%, BAP, 1 mg/l (Sopory, Jacobsen and Wenzel, 1978; Wenzel and Uhrig, 1981). Donor plants for anther culture can be grafted on to tomato to prolong flowering (Wenzel and Uhrig, 1981).

Using such techniques Sopory, Jacobsen and Wenzel (1978) achieved microspore embryogenesis in 35% of cultured dihaploid anthers. Of 22 embryos examined cytologically, seven were found to be monohaploid, 14 had 24 chromosomes and one contained the full tetraploid complement. In a more exhaustive study, Wenzel and Uhrig (1981) produced 6000 clones from cultured dihaploid anthers: about 90% of these appeared to have doubled up spontaneously in culture to give fertile double monohaploid clones ($2n = 2x = 24$); the remainder were monohaploids. Hybrid donor clones carrying resistance genes to *Globodera rostochiensis* and potato virus Y (PVY) yielded homozygous resistant double monohaploid lines.

The main limitation of anther culture is that progress so far has been restricted to few genotypes, although attempts to breed in responsivity (i.e. higher recovery from anther culture) have met with some success (Uhrig, 1983).

PROTOPLASTS

Potato plants can be regenerated from wall-less cells (or protoplasts) (*Figure 3*). This is very important for direct genome manipulation by mutation, fusion or transformation, where the aim, to obtain plants in which all cells contain the altered genotype, can be achieved most satisfactorily through regeneration from single cells. These applications are discussed in some detail later.

In general terms, to obtain viable leaf protoplasts of potato it is necessary to control leaf growth conditions carefully. Plants must either be grown in controlled-environment cabinets with specific light, nutrient and humidity regimes, or else as shoot cultures. After plasmolysis the protoplasts are released by enzyme digestion of the cell wall (*Figure 3a*) and then protected from bursting by provision of a suitable osmotic environment. They are washed and cultured in an appropriate medium which allows the synthesis of new cell walls followed by cell division. Repeated divisions result in the formation of colonies (*Figure 3b,c*). When these are large enough to be visible to the naked eye they are transferred to a solid medium on which further growth occurs to form callus (*Figure 3d*). This is followed by transfer to media that induce differentiation and shoot emergence (*Figure 3e*) and then to a 'rooting' medium on which roots are established on the shoots (*Figure 3f*). The yield of regenerated plants may be 5% of the protoplasts originally cultured (Nelson, 1983). Plants can be maintained and multiplied

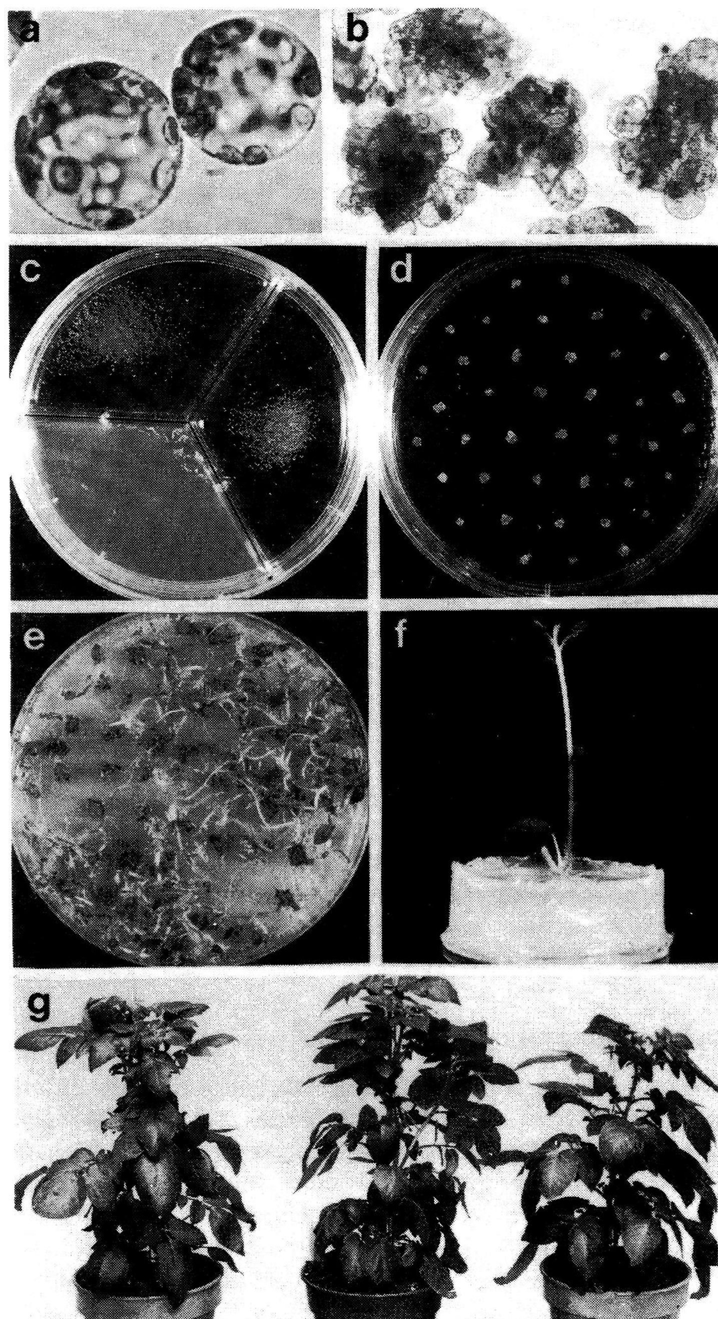

Figure 3. Plant regeneration from potato protoplasts. (a) isolated leaf protoplasts; (b) protoplast-derived colonies; (c) protoplast-derived colonies in divided dishes; (d) colonies on agar; (e) shoot regeneration from colonies; (f) protoplast-derived plantlet; (g) regenerated plants (cv. Majestic) in soil.

as shoot cultures or they can be transferred to soil (*Figure 3g*). The whole procedure may take 4–6 months.

Successful plant regeneration has now been achieved for a wide range of cultivars and breeding lines of US and European origin. These include Russet Burbank, Katahdin, Kennebec, Bison, Atlantic, Primura, Maris Piper, Desirée, Majestic, Pentland Crown, King Edward, Maris Bard, Bintje, Feltwell, Foxton, Ukama, Spunta and several others (Shepard and Totten, 1977; Gunn and Shepard, 1981; Thomas, 1981; Bokelmann and Roest, 1983; Jones *et al.*, 1983; Nelson, 1983; Sree Ramulu, Dijkhuis and Roest, 1983; Creissen and Karp, 1985; Haberlach *et al.*, 1985; Foulger and Jones, 1986). In addition, plants have been regenerated from dihaploid potatoes (Binding *et al.*, 1978; Wenzel *et al.*, 1979; Haberlach *et al.*, 1985), and several other *Solanum* species. The latter include *S. brevidens* (Nelson, Creissen and Bright, 1983; Haberlach *et al.*, 1985); *S. phureja* (Creissen, 1984; Haberlach *et al.*, 1985), *S. chacoense* (Butenko *et al.*, 1977), *S. dulcamara* (Binding and Nehls, 1977), *S. etuberosum* and *S. penellii* (Haberlach *et al.*, 1985). In an extensive screen, Haberlach *et al.* (1985) were able to regenerate plants from 22 of the 36 lines tested. However, procedures are not necessarily routine, and methods used vary from one laboratory to another. Careful study to optimize the culture and regeneration of three cultivars clearly showed that the efficiency of regeneration is genotype related, and different genotypes require different culture conditions (Shepard, 1982a; Foulger and Jones, 1986).

Consequences of a callus phase

Tissue culture techniques involving propagation via meristems or axillary buds are generally characterized by genetic stability (D'Amato, 1978). In contrast, regeneration of plants from cultured protoplasts, explants and organs involves adventitious shoot formation from callus, and it has been known for some time that instabilities can occur in disorganized tissue (Bayliss, 1980).

CYTOLOGICAL INSTABILITY

There are numerous reports in the literature of changes in both chromosome number and structure in calluses of various origins and in plants regenerated from cultures *in vitro* (D'Amato, 1978; Constantin, 1981; Wersuhn and Dathe, 1983). This evidence raises the issue of whether chromosome instability will be a problem in the use of tissue culture steps in potato breeding.

In early studies of potato plants regenerated from protoplasts of the American cultivar Russet Burbank, Shepard, Bidney and Shahin (1980) found no changes in the chromosome number of five clones. In later work (Shepard, 1982b) the presence of aneuploidy was surmised from the poor morphology of some of the regenerants and such plants were discarded. Since then, both structural and numerical chromosome changes have been identified (Gill, Kam-Morgan and Shepard, 1985, 1986).

An early cytological study of protoplast-derived plants of British cultivars Maris Bard and Fortyfold provided clear evidence that numerical chromosome variation could be extensive. Only 4% of the regenerants were normal euploids in cultivar Maris Bard and 30% in cultivar Fortyfold. The aneuploid regenerants had either lost or gained a few chromosomes (for example, 2n = 47, *Figure 4a*) or else resulted from chromosome doubling followed by

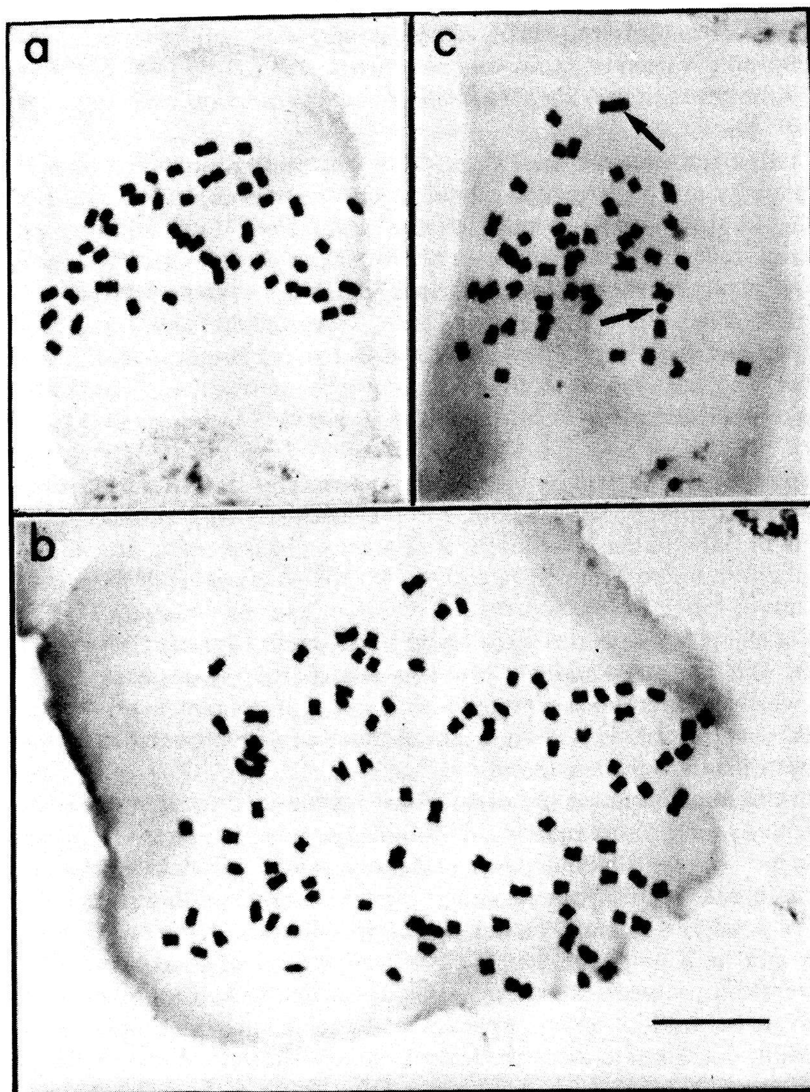

Figure 4. Chromosomes in root-tip cells of protoplast-derived potato plants; (a) aneuploid, 2n = 47; (b) regenerant with a high chromosome number, 2n = 93; (c) euploid regenerant (2n = 4x = 48) heterozygous for a translocation. A segment from one chromosome has been translocated on to another, generating one chromosome which is smaller than the rest of the complement and one which is larger (arrows). (Scale bar 10 μm).

loss, and contained very high chromosome numbers ranging from 2n = 72 up to 2n = 96 (*Figure 4b*). Plants with high numbers were grossly abnormal in morphology, whereas aneuploids at the tetraploid level were not always distinguishable from normal, euploid regenerants (Karp *et al.*, 1982).

Similar variation has been reported in other cultivars (Sree Ramulu, Dijkhuis and Roest, 1983; Creissen and Karp, 1985). Variation in the frequency of normal plants (5–60%) probably arises from cultivar differences in response and from the use of different media and culture methods. Frequencies of normal tetraploids have been increased up to 60% in recent studies (Fish and Karp, 1986). There is also clear evidence of structural chromosome variation (Creisson and Karp, 1985; Fish and Karp, 1986; Gill, Kam-Morgan and Shepard, 1986) including deletion and translocations (*Figure 4c*).

Potato plants regenerated from dihaploid protoplasts were uniform in morphology and nearly all tetraploids, only two aneuploids being present among 48 regenerants (Wenzel *et al.*, 1979). The different nature of this variation reflects the difference in ploidy of the source material. Similarly, 52% of plants regenerated from diploid *Solanum brevidens* protoplasts were found to have doubled in ploidy, 24% were diploid, and the remainder aneuploid at the tetraploid level (Nelson, Karp and Bright, 1986). Variation of this kind has also been found in protoplast-derived regenerants of the diploid medicinal plant *S. khasianum* (Kowalczyk, Mackenzie and Cocking, 1983).

In contrast to the extensive chromosomal changes that characterize potato plants regenerated from protoplasts, regeneration from cultured explants results in only minimal variation. In a survey encompassing regenerants of three different cultivars (Champion, Myatts Ashleaf and Desirée) and explants of four different sources (leaves, stem, rachis and tuber), on average 87% of the plants screened were found to be stable euploids (Wheeler *et al.*, 1985). This improved stability probably reflects the greater ease and speed with which regenerants can be obtained from explants compared with protoplasts. One possible exception is that of tuber explants, from which four out of seven plants were aneuploid.

Chromosome variation in potato plants regenerated from *in vitro* culture, particularly of protoplasts, is undesirable in schemes aimed towards the production of plants modified in specific ways, and it will clearly be necessary to screen out variant types resulting from these regeneration procedures. It may be possible to identify factors causing instability and to remove or reduce them and both genotype-dependent differences in response and different regeneration protocols should be studied. Chromosome instability may not, however, be without some application: it can be exploited as a means of achieving doubling, as described in the following section and of obtaining introgression in hybrids, as demonstrated in cultures of *Triticale* (Armstrong, Nakamura and Keller, 1983).

MANIPULATION OF PLOIDY

Chromosome doubling in a callus phase can be exploited as a means of obtaining polyploid plants (Murashige and Nakano, 1966). In potato, which is already tetraploid, this can be most usefully applied for the production of doubled-up genotypes from dihaploids and monohaploids. In the case of monohaploids there are two possible doubling steps, resulting in the formation of homozygous tetraploid plants (Hermsen and Ramanna, 1981). The first is to the double monohaploid (DMH, $2n = 2x = 24$) and the second from the DMH to the doubled DMH (DDMH, $2n = 4x = 48$).

Doubling via tissue culture has been successfully carried out in an *S. etuberosum* × *S. pinnatisectum* hybrid by regeneration from rachis and petiole explants, where out of 112 plants screened cytologically, 84% had completely doubled and no chimaeras were present (Hermsen *et al.*, 1981). For dihaploid potato, Jacobsen (1981) reported a high frequency of doubled genotypes from cultured leaf explants and again periclinal chimaeras were not found. Recently, Karp *et al.* (1984) demonstrated that regeneration from cultured leaf segments (*Figure 2*) can be used effectively as a means of obtaining homozygous tetraploid plants from monohaploid lines. A single leaf regeneration cycle resulted in almost total recovery of double monohaploids, only one plant out of 11 having remained monohaploid in constitution (*Figure 5a*). When leaves from these plants were passed through a

Figure 5. Leaf morphology and chromosome constitution in potato monohaploids and their double counterparts. The leaves shown are the fifth youngest and an older fully expanded leaf. Chromosomes are from root-tip preparations. (a) monohaploid $2n = x = 12$; (b) doubled monohaploid $2n = 2x = 24$.

second regeneration cycle. 50% of the plants recovered had doubled again to the homozygous tetraploid level and the others remained DMH ($2n = 2x = 24$, *Figure 5b*). A similar regeneration procedure using dihaploid leaf pieces also gave a good recovery (60%) of doubled plants.

Chromosome doubling can be achieved using colchicine treatment, but the method is laborious, recovery of doubled genotypes is limited and mixoploidy frequent (Frandsen, 1976; Ross, Dionne and Hougas, 1967; Hermsen and De Boer, 1971). The advantages of using tissue culture are that large numbers of plants can be obtained simply and rapidly, and the proportion of mixoploids or aneuploids is minimal.

SOMACLONAL VARIATION

Plants propagated via conventional stem cuttings or through axillary bud micropropagation are to a large extent uniform among themselves, in accordance with their characteristic genetic stability referred to earlier. Variants, or sports, do arise at low frequency but these are dealt with in breeding programmes by roguing. In contrast, when adventitious shoots are formed after cells have passed through a disorganized callus phase, the resulting plants may show a wide range of phenotypic changes. This phenomenon has recently been named 'somaclonal' variation (Larkin and Scowcroft, 1981), following the demonstration that it may involve agronomically useful traits.

The question at the centre of this renewed interest is whether this source of variability is of use to plant breeders. In order to address this question it is necessary to make a thorough assessment of the type of changes that can be found and their frequency.

The nature and extent of somaclonal variation

A substantial body of work with sugar-cane has documented stable changes, particularly in disease-resistance characters, which have arisen in plants regenerated from immature leaves (Heinz *et al.*, 1977; Larkin and Scowcroft, 1983) and at least one tissue-culture-derived sugar-cane cultivar has been released and is widely grown.

In potato the picture is not yet as complete. Hundreds of clones derived from cultured protoplasts of cv. Russet Burbank have been examined for many agronomic characters over several years by Shepard and his collaborators. Clones were identified with changes in plant geometry, photoperiod response and leaf variegation as well as in tuber yield, shape, number and depth of setting (Shepard, 1980; Shepard, Bidney and Shahin, 1980; Secor and Shepard, 1981). Furthermore, interesting changes were found in the response of some clones to late blight (*Phytophthora infestans*) and early blight (*Alternaria solani*) when challenged by crude culture filtrates or live fungus (Matern, Strobel and Shepard, 1978; Shepard, Bidney and Shahin, 1980).

Analysis of potato clones derived from callus rather than protoplasts has also provided good evidence that potentially useful variation arises during this technically simpler process. In an experiment designed to test the effect

of X-irradiation, van Harten, Bouter and Broertjes (1981) found that 30% of the non-irradiated control plants regenerated from leaf or rachis pieces of cv. Desirée showed evidence of spontaneous mutation, including, for example, some changes in tuber colour. In an extensive analysis of several hundred clones regenerated from leaf, rachis or stem pieces of Desirée, changes in tuber colour, tuber number and extent of scab (*Streptomyces scabies*) infection have been observed (Wheeler *et al.*, 1985; Evans *et al.*, 1986). Statistical evaluation by principal component analysis indicated that approximately one-half of 346 field-grown clones differed from the control plants (*Figure 6*).

The frequency of individual variant types is difficult to determine precisely, but can clearly be very high in some instances. In the case of sugar-cane only 23% of the 235 callus-derived clones had the same response to Fiji disease as the controls, and over one-half of the clones were more resistant. Similar high frequencies of change were found in response to eyespot and downy mildew (Heinz *et al.*, 1977; Larkin and Scowcroft, 1983). In potato, 500 protoplast-derived clones were analysed for differences in response to a crude toxin preparation of *Alternaria solani*. Five clones were identified with some degree of resistance and four of these were more resistant to infection by the fungus, a trait that was carried through two tuber generations (Matern, Strobel and Shepard, 1978; Shepard, Bidney and Shahin, 1980). Similarly, 20 clones, out of 800 tested, were resistant to *Phytophthora infestanus* race 0, and at least four of these were also resistant to races 1, 2, 3 and 4 in a mixed

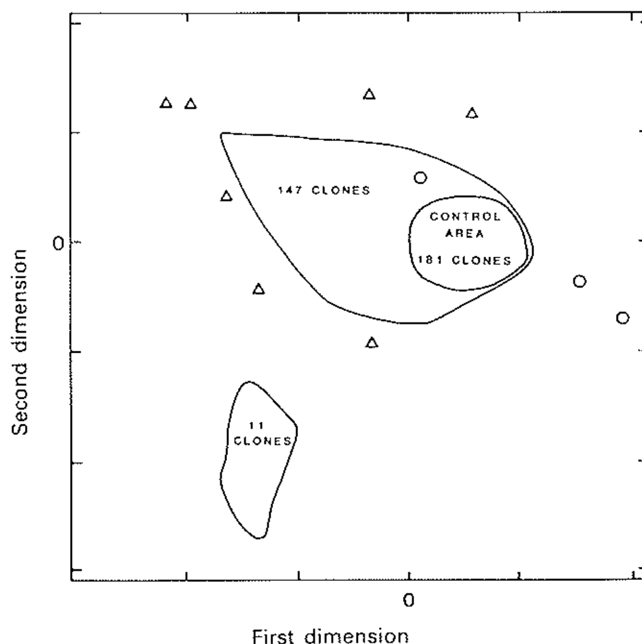

Figure 6. The distribution of 346 clones of potato regenerated from cv. Desirée along the first two dimensions after principal component analysis. The majority of clones were within the hatched areas. Individual outlying clones of controls (o) and regenerants (△) are indicated.

infection (Shepard, Bidney and Shahin, 1980). Although these frequencies appear to be quite high (1–2%), in both cases the clones were tested only after discarding an unspecified number of original regenerated shoots, which, as explained in an earlier section, were probably aneuploids. The actual frequency of resistant plants may therefore be lower.

Sixty-five protoplast-derived clones of cultivar Russet Burbank were tested in sextuplicated trials and compared with two control clones. Statistically significant differences were found in each clone in at least one and up to 17 of the 22 characters assessed (Secor and Shepard, 1981), again suggesting a high general frequency of variation, although in nine of the characters no differences were found at all.

Scab response tested in several hundred explant-derived clones of cultivar Desirée over two field generations showed a different, and rather intriguing pattern of variation. In this case the response of the entire regenerant population was found to have shifted towards better resistance (i.e. lower scab scores, *Figure* 7), a change that has been stable for three years in the field (Evans *et al.*, 1986). In contrast, 184 of these same clones showed no change in susceptibility response when tested with *Globodera pallida*.

The potential application of somaclonal variation to potato breeding

The presence of variants with increased yield or resistance to disease indicates the potential importance of somaclonal variation for plant breeding. It could be most useful in situations where modifications in specific traits are desired in otherwise well-adapted cultivars. However, as yet, no new potato cultivars have been released from a somaclonal variation programme and a number of problems need to be addressed before this tissue culture phenomenon can be placed in a practical breeding context.

The first problem lies in understanding the cause of the variation. We have already described the evidence that chromosomal changes can occur

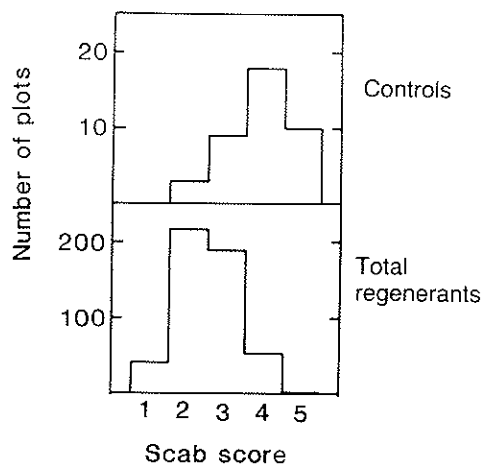

Figure 7. The distribution of tuber scab scores (range 1 (=0%) – 5 (=75%) of surface affected) in plants grown in field plots at Broughshane, N. Ireland, in 1983. The plots contained plants of Desirée controls and clones regenerated from leaf and rachis pieces, all grown at one site.

among the regenerated plants and many gross changes in phenotype can be accounted for in this way. However, apparently chromosomally normal plants still exhibit phenotypic variability and it is commonly this that is referred to by the term 'somaclonal variation' and that is of interest for plant breeding. Both genetic and non-genetic changes have been implicated in its origin (Larkin and Scowcroft, 1981). There is evidence that some variation results from alterations in the genome, including single gene mutations (Barbier and Dulieu, 1980; Edallo *et al.*, 1981; Evans and Sharp, 1983), changes in DNA amount (Prat, 1983), DNA transposition and cytoplasmic changes (Gengenbach *et al.*, 1981). Changes at the DNA level have recently been reported in potato. Two out of 12 protoplast-derived clones studied by Landsmann and Uhrig (1985) were found to have deficiences in 25–S ribosomal DNA, while among protoplast-derived clones of cv. Russet Burbank, variation was detected in mitochondrial, but not in chloroplast, DNA (Kemble and Shepard, 1984). The instability of some of the changes and the high frequencies of others suggest that not all of the variation may have a genetic basis. The nature of these changes has yet to be determined, but there is increasing evidence that differences in DNA methylation may be involved (Brown and Lörz, 1986).

Another problem lies in the spectrum of changes that are available and their unpredictability. Results so far suggest that not all changes are possible. For example, whereas tuber variants with white skins were found among regenerants of Desirée (normally red-skinned), no clones with coloured tubers have been observed among the many hundred regenerants screened from white-skinned cultivars (Wheeler *et al.*, 1985). In addition, traits which change, do so at highly variable frequencies. These characteristics present breeders with the problem of having to deal with vast numbers of plants without any guarantee of success.

A strategic decision is required regarding the proportioning of effort to be devoted to somaclonal variation as against conventional breeding. The indications are that explants are a more useful source of variation, as they are less labour intensive and quicker to work with than protoplasts, and show considerably less chromosome variation. However, large numbers of good clones which show no change from the parent cultivar are to be expected and it will often be necessary to screen for desired characteristics in large plots with good replication.

A new cultivar will need to be distinct to be successful and this may be a problem if the improved character is not useful for identification. Flower colour and leaf shape variants which are of no intrinsic benefit may come to be of use in this regard. The logical extension of this is to ask whether a neutral change, such as flower colours, could allow a competing breeder to produce and market an otherwise unchanged successful cultivar, such as Maris Piper.

Areas of development

The ability to culture cells either singly, as protoplasts, or in tissues or organs and the subsequent regeneration of plants provides a unique experimental

system for a number of developing areas aimed at more direct manipulation of the potato genome. Three of these areas—mutant selection, protoplast fusion and transformation—are discussed in this section.

MUTANT SELECTION

Mutants are useful for unravelling biochemical pathways, as genetic markers for crosses or transformation and fusion studies, and may also be of direct use in an applied sense. Considering the importance of the potato crop there is relatively little work in genetics at the whole-plant level (Howard, 1970) and very few mutants have been identified. Tissue culture techniques provide an opportunity for selection of mutants *in vitro*. Mutagenic agents, such as X-rays, UV light or chemical mutagens, can be applied to cultured plant cells or, conversely, spontaneous mutants could be screened in culture *in vitro* on selective media or selective growing conditions. Such approaches are not without hazards, however. A major problem is that the expression of mutant phenotypes in culture is not always assured in the regenerated plants. Resistance or susceptibility in host–pathogen interactions have been stably maintained in some instances, as for example in blackshank disease of tobacco (Deaton, Keyes and Collins, 1982) but more often it is lost. Furthermore, the action of some herbicides may be different in culture compared with that in plants (Zilkah and Gressel, 1977).

Mutant selection *in vitro* is an approach largely unexplored in potato, but illustration of the potential of this method can be supplemented by examples from other plant species. A potato cell line resistant to 5-methyltryptophan (5MT) has been used to demonstrate the presence of two possible isoenzymes of anthranilate synthase (EC 4.1.3.27) (Carlson and Widholm, 1978)—the first regulatory enzyme unique to tryptophan biosynthesis. Further 5MT-resistant cell lines were isolated by Jacobsen, Visser and Wijbrandi (1985). The cells accumulated free tryptophan (Carlson and Widholm, 1978) and phenylalanine and tyrosine (Jacobsen, Visser and Wijbrandi, 1985) demonstrating the possibility of manipulating the free amino acid pools of potato cells, as has been achieved for barley and maize seeds (Bright *et al.*, 1982; Hibberd and Green, 1982). Jacobsen (1986) also isolated a potato line resistant to 5-(2-aminoethyl)cysteine (AEC). The possibilities of increasing and decreasing sugars, amino acids and starch in the intact potato plant by mutation of key enzymes remains to be explored. Such studies could provide valuable insight into the biochemistry, physiology and processing of potato, and should be aided considerably by the transformation studies discussed later.

It is possible to identify a few characters which could be used as genetic markers in potato. This includes 5MT resistance described above. Potato cells lacking an auxin requirement for growth *in vitro* have been identified (Widholm, 1977) and can be obtained from galls transformed by *Agrobacterium tumefaciens* (Ooms, Karp and Roberts, 1983). Albino mutants in a number of cultivars would also be very useful. They might be expected to arise at quite high frequency following mutagenesis and regeneration from

monohaploid leaves, but none have been observed so far out of several hundred shoots regenerated after UV-irradiation (R. Risiott and S. W. J. Bright, unpublished). Albino cells from a variegated protoplast-derived 'somaclone' of cultivar Russet Burbank have been isolated and used in protoplast fusion (Shepard, Bidney and Shahin, 1980; Shepard *et al.*, 1983), as described later.

Mutants resistant to herbicides or disease may be of direct practical value. Fertile tobacco plants resistant to sodium chlorate (Muller, 1983), picloram (4-amino-3,5,6-trichloropyridine-2-carboxylic acid) (Chaleff, 1983), chlorsulfuron (1-(2-chlorophenylsulphonyl)-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)) and sulfometuron methyl (methyl ester of 2-[3-(4,6-dimethylpyrimidin-2-yl)ureidosulphonyl] benzoic acid) (Chaleff and Ray, 1984) have been selected from cultivated cells. In principle, no barrier prohibits this strategy from being used in potato. However, it requires identification of a suitable herbicide which should inhibit cells in the same way as plants and which would actually be used in the field should its selectivity be improved. Single gene traits such as this are also candidates for gene transfer into potato by transformation.

The addition of toxins or culture filtrates of pathogens has been used for screening of potato plants for resistance (Matern, Strobel and Shepard, 1978; Shepard, Bidney and Shahin, 1980), but their application to selection *in vitro* has proved more difficult. The principle has been demonstrated in maize where callus and plants resistant to T-toxin of *Helminthosporium maydis* were regenerated from toxin-susceptible cells cultured in the presence of the toxin (Gengenbach, Green and Donovan, 1977). Resistance was correlated with DNA changes in the mitochondria of regenerated plants (Umbeck and Gengenbach, 1983).

In potato, there is one result which is both interesting and challenging. Of 41 000 calluses from four dihaploid genotypes plated on media containing culture filtrates of various races of *Phytophthora infestans*, 36 (0·01%) survived for at least two transfers. The frequency was slightly higher after X-irradiation (7/1400 = 0·5%). An unspecified number of these resistant calli regenerated plants, and callus from these leaves was often more resistant when rechallenged (Behnke, 1979). Later, 34 plants from 10 selected calli were compared with 15 plants from unselected calli. Lesion diameters and numbers of sporangia were measured for 4 days after inoculation of excised leaflets with sporangia of mixed pathotypes of *P. infestans*. Lesion diameters were 25% smaller in the resistant regenerants but numbers of sporangia produced were not different (Behnke, 1980). A pathological characterization of the resistance in these plants compared with known sources of resistance would be useful in assessing the breeding value of such selected variants.

There will undoubtedly be further development of the tissue culture approach to mutant selection. Practical benefits may appear on a more long-term scale. Perhaps the most exciting mutants to be produced in the near future will be those with genes derived from other plant species or other sources introduced by transformation.

PROTOPLAST FUSION

The liberation of protoplasts from their rigid cell walls renders them amenable to fusion. Together with the capacity to regenerate plants from these protoplasts, this provides a unique opportunity for production of somatic hybrids. The attraction of the procedure is threefold: (1) it offers to extend the possibility of hybrid formation to widely unrelated forms unable to interact sexually; (2) it offers an asexual means of effecting gene transfer either of whole genomes, or of partial genomes; (3) traits can be transferred without the need for detailed knowledge of their precise genetic basis.

Producing somatic hybrids

Protoplasts may be fused by chemical or electrical procedures (Kao *et al.*, 1974; Zimmermann, 1982). The most frequently used method is a combination of polyethylene glycol (PEG) to agglutinate the protoplasts followed by elution with Ca^{2+} ions at high pH (Kao and Michayluk, 1974) to allow membrane coalescence and cytoplasmic mixing (*Figure 8a*). Salt mixtures (Binding, 1974), have also been utilized. Additives to PEG such as dimethyl sulphoxide (Menczel *et al.*, 1981) or lectins (Glimelius, Wallin and Ericksson, 1978) have improved fusion frequencies. These techniques are discussed in more detail by Evans (1983) and Fish, Karp and Jones (1987).

More recently, physical methods of fusion using electric fields have been developed (Zimmerman, 1982). Protoplasts, when introduced into a high-frequency alternating electric field (0·1–1 MHz) in a low-conducting medium (e.g. mannitol), between parallel electrodes 0·1–1·0 mm apart, act as dipoles and are attracted to each other like magnets along the strongest field lines. This 'dielectrophoresis' results in the formation of double or multiple 'pearl chains'. Once the protoplasts are in contact, a high frequency of fusions can be achieved by disrupting the protoplast membranes with a short direct-current pulse. By control of protoplast density, method of introduction and fusion conditions it is possible to obtain a much higher frequency of one-to-one fusions than with PEG, with production of viable heterokaryons (*Figure 8c,d*) (Tempelaar and Jones, 1985a,b; Tempelaar *et al.*, 1987) and this approach could supersede the chemical fusion methods. The advantage of electrofusion over chemical methods is that conditions can be optimized rapidly, and this is particularly useful for sensitive mesophyll protoplasts, such as potato.

Heterokaryons, produced by fusion of different genotypes, must be selected from a mixture also containing homokaryons (produced by fusion of identical protoplasts) and unfused protoplasts. Complementation between mutant donor lines has been used to achieve this. For example, fusion of non-allelic recessive albino mutants of *Datura* and tobacco gave rise to green hybrid callus (Schieder, 1977), and AEC- and 5MT-resistant potato lines have been used for selection of hybrid lines in potato (de Vries *et al.*, 1987). Complementation can also be achieved metabolically, by the use of chemically inactivated parents (Wright, 1978; Medgysey, Menczel and

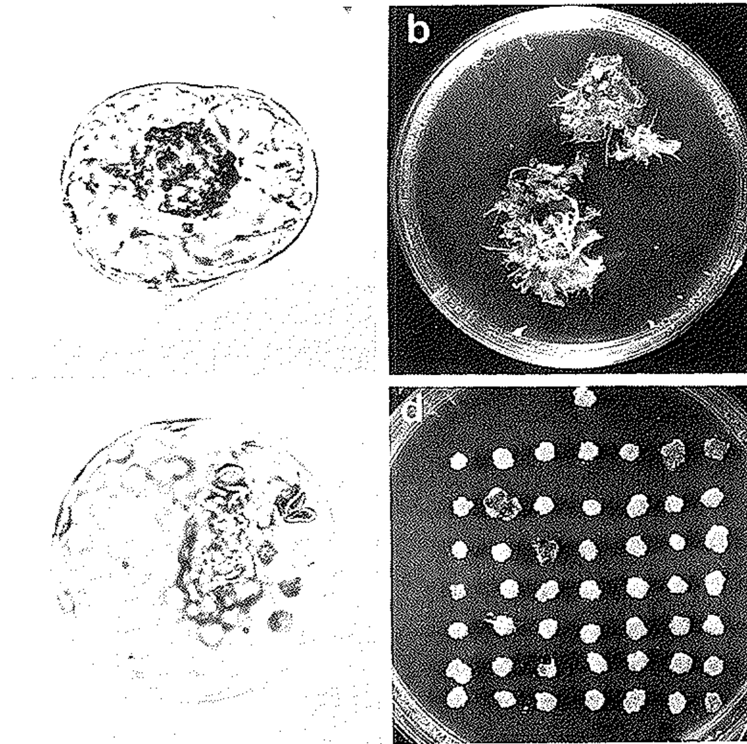

Figure 8. (a) Heterokaryon obtained by fusing *S. tuberosum* mesophyll protoplast with *N. sylvestris* suspension protoplast using PEG, 48 h after fusion. Heterokaryon isolated by micropipette. Note cell wall formation (shape not spherical) and chloroplasts from *S. tuberosum* migrating to surround the nuclei. (b) Shoots on callus obtained from isolated heterokaryons as in (a). (c) Heterokaryon obtained (partners as in (a)) by electrofusion 2 h previously. The chloroplasts of *S. tuberosum* are clearly visible. (d) Colonies derived from protoplast following electrofusion.

Maliga, 1980; Sidorov *et al.*, 1981). Other methods of heterokaryon selection include manual isolation using micropipettes (Gleba and Hoffmann, 1980) and the utilization of known differences in the culture responses of different parents (Evans, 1983). The heterokaryons may require culture with nurse cells to avoid the problem of poor growth at low cell densities. Once established, hybrid colonies can be handled individually and cultured to regenerate whole plants.

Somatic hybrids in potato

Protoplast fusion technology is now being applied widely to potato. Early work on somatic hybridization involved fusion of *S. tuberosum* protoplasts with those of *Lycopersicon esculentum* (Melchers, Sacristan and Holder, 1978); Shepard *et al.*, 1983), *S. chacoense* (Butenko and Kuchko, 1980), *S. nigrum* (Binding *et al.*, 1982) and *Nicotiana tabacum* (Skarzhinskaya, Cherep

and Gleba, 1982). Most of these studies involved suspension protoplasts as one partner, and from what we now know of the cytological abnormalities that rapidly accumulate in such cultures, it is not surprising that many of the somatic hybrid plants produced were aneuploid.

More recently, a series of hybrid plants have been produced by chemically fusing potato protoplasts with those of *S. brevidens*. The aim has been to transfer resistance to potato leaf roll virus (PLRV) and to blight (*Phytophthora infestans*) from this diploid wild species into potato. Thus Barsby *et al.* (1984) fused an albino tetraploid potato and *S. brevidens* protoplasts, and obtained some hexaploid and more high chromosome number aneuploid plants. The most extensive programme has been carried out at the University of Wisconsin, where somatic hybrid plants have been produced using mesophyll protoplasts between both tetraploid and dihaploid *S. tuberosum* plus *S. brevidens*, to produce hexaploid and tetraploid hybrids respectively (Austin, Baer and Helgeson, 1985; Austin *et al.*, 1985; Helgeson *et al.*, 1986). These workers demonstrated expression of resistance to PLRV and blight in hybrid plants. Extensive field testing of hexaploid hybrids was also carried out (Austin *et al.*, 1986). Most hybrids flowered, had viable pollen and set tubers, and a wide range of variation in morphological characters was observed. In general, hybrids grew more vigorously than parental types. Further studies indicated that, except for a low level of selfing, pollen of 4x and 6x hybrids was ineffective in pollinations (Ehlenfeldt and Helgeson, 1987). However, as female parents in crosses, the 6x hybrids in particular crossed well with 4x testers, and produced progeny at the 5x level. Further crosses (5x × 4x) are expected to yield tetraploid plants (J. P. Helgeson, personal communication). Thus reasonable levels of female fertility can be obtained in these somatic hybrids.

These results have been repeated using both chemical (*Figure 9*) and electrofusion (Fish, Karp and Jones, 1987; N. Fish and M. G. K. Jones, unpublished), and have confirmed transfer of PLRV resistance from a different

Figure 9. Tetraploid somatic hybrid plant (centre) produced by fusing protoplasts from *S. brevidens* (left) and a dihaploid *S. tuberosum* (right).

genotype of *S. brevidens* into tetraploid hybrids. Puite, Van Wikselaar and Verhoeven (1986) also reported the production of somatic hybrids by electrofusion of dihaploid potato and *S. phureja* protoplasts.

The feasibility of another practical application of fusion, the synthesis of tetraploid hybrids (i.e. intraspecific fusion) has also been elegantly demonstrated by Austin *et al.* (1985). They fused protoplasts from one dihaploid line with gold-yellow tuber flesh with those of a dihaploid line with mottled red tuber flesh. Somatic hybrid plants produced remarkably pigmented tubers, with black-purple skin and dark purple flesh.

The use of somatic hybridization in breeding

With the successful production of hybrid plants at the tetraploid and hexaploid levels, both for inter- and intraspecific fusions, the number of practical applications of fusion technology are increasing. Thus, as has been outlined above, characters from wild species that are sexually incompatible may be introduced in *S. tuberosum* via fusion. However, perhaps the most useful application is in combining potato breeding at the dihaploid level with intraspecific fusion. Breeding at the dihaploid level is easier than at the tetraploid level for a variety of reasons, but there are also some disadvantages (e.g. reduced fertility). Somatic hybridization provides the opportunity to combine dihaploid lines selected for different agronomic characters. This bypasses dihaploid sterility problems, and heterosis could be maximized without the reassortment of dominant characters at meiosis (Fish, Karp and Jones, 1987). It is important to be aware of two factors relevant to achieving this end—that individual agronomically useful genotypes may not respond to standard tissue culture regimes, and that selection of hybrids is more difficult than for interspecific fusions. Current work (Fish, Karp and Jones, 1987) has shown that with careful attention the first factor can usually be resolved, and the high-fusion frequencies that now can be obtained using electrofusion (Tempelaar and Jones, 1985a,b; Tempelaar *et al.*, 1987) can reduce problems of selection if a high proportion of colonies grown on after fusion are, in fact, hybrid.

Other approaches to genetic manipulation include the irradiation of donor protoplasts with useful characters, to fragment their genomes, followed by fusion to tetraploid acceptor protoplasts. The aim here is to induce partial genome transfer without otherwise altering an existing cultivar.

Work to transfer specific biochemical traits into potato in this way is in progress. For example, heterokaryons formed by fusion between protoplasts of *N. sylvestris* resistant to AEC and protoplasts of *S. tuberosum* have been isolated and grown to form colonies and shoots (*Figure 8a,b*), but irradiation of the AEC donor failed to yield AEC-resistant hybrids (Foulger *et al.*, 1985), although success has been achieved with this approach for other species.

Protoplast fusion also provides a means of transferring cytoplasmic traits into another genomic background. This has been achieved for transfer of cytoplasmic male sterility into rape (Pelletier, Primard and Vedel, 1983),

and attempted for transferring chloroplast-encoded atrazine resistance from *S. nigrum* into potato (Binding *et al.*, 1982; Gressel, Cohen and Binding, 1984).

An interesting feature of somatic hybrids, including those of potato, is that they normally exhibit a range of phenotypes because individual plants may possess chloroplasts from either parent, and recombined mitochondria. To this may be added a background of somaclonal variation. Thus new combinations of genes may be obtained after fusion even from sexually compatible parents. This result indicates that the somatic hybridization approach should not be restricted solely to production of hybrids with sexually incompatible species, but has a potentially wider contribution to breeding.

TRANSFORMATION

There have been great advances in the ability of molecular biologists to identify, isolate, and manipulate specific DNA segments in bacterial plasmids. Increasing numbers of genes from bacteria, animals and plants have been treated in this way. With the advent of these techniques, a potential has emerged for crop improvement by transformation, or direct incorporation of DNA into a recipient genome. The aim is the stable integration of specific isolated genes into the genome of a plant and their expression in a controlled manner. This promises a potent means of producing potatoes with specifically modified genotypes and phenotypes. Recent progress in whole-plant transformation studies using the naturally occurring transformation system of *Agrobacterium* and newly developed regeneration procedures has already shown that specific gene transfer has real potential in potato.

Agrobacterium *as a tool for gene transfer*

Agrobacterium tumefaciens is a common soil bacterium which, upon infection of plant wounds, induces crown gall tumours at the site of infection in dicotyledonous plants. The tumours are characterized by the presence of a plasmid DNA fragment of bacterial origin which is transferred and stably integrated into apparently any of the plant chromosomes (Hooykaas and Schilperoort, 1984). This fragment, T-DNA, is expressed and causes hormonal disturbances in the transformed plant cells. Most notable of these are the enhanced endogenous concentrations of the plant hormones auxin and cytokinin. In a similar way, the related bacterium *A. rhizogenes* causes prolific root development, or 'hairy-root' symptoms caused by a different type of T-DNA present in the transformed roots (White *et al.*, 1982).

The basic transformation system developed using *Agrobacterium* involves replacement of the natural tumour-inducing (in the case of *A. tumefaciens*) or hairy-root-inducing (in the case of *A. rhizogenes*) genes by bacterial genes conferring resistance to antibiotics such as kanamycin and hygromycin. These antibiotic-resistance genes have been isolated and engineered *in vitro* (e.g. Bevan, Flavell and Chilton, 1983) into suitable vector plasmids. The engine-

ered plamids are transferred by *Agrobacterium* into the plant cell, using the same mechanism as for the replaced genes. Transformants can then be selected by growing the cells on medium containing the antibiotic. In principle, any plant cell susceptible to *Agrobacterium* can be transformed in this way. Provided suitable regeneration systems are available, a whole transformed plant can then be regenerated containing the introduced genes in all its cells. Further details of *Agrobacterium*-based and non-*Agrobacterium*-based transformation systems can be found in Hooykaas and Schilperoort (1984) and Nester *et al.* (1984).

Transformed potato plants

Efficient production of crown gall and hairy root tissues has been obtained by infecting wounded potato shoots cultured *in vitro* with bacterial suspensions (*Figure 10a*). In a series of experiments aimed at developing general transformation/regeneration systems, potato plants were regenerated from both crown galls and hairy roots induced on commercially grown cultivars (Ooms *et al.*, 1983, 1985, 1986b). The regenerated plants contained the tumour-inducing and hairy-root-inducing T-DNA genes, respectively. These were expressed at different levels and in different organs of the transformed plants (Ooms *et al.*, 1986b). In the case of cultivar Maris Bard transformed with *A. tumefaciens*, the transferred T-DNA genes caused most notably an increase in endogenous cytokinins. The resultant plants showed characteristic alterations in morphology and development. In particular, the photoperiodic require-

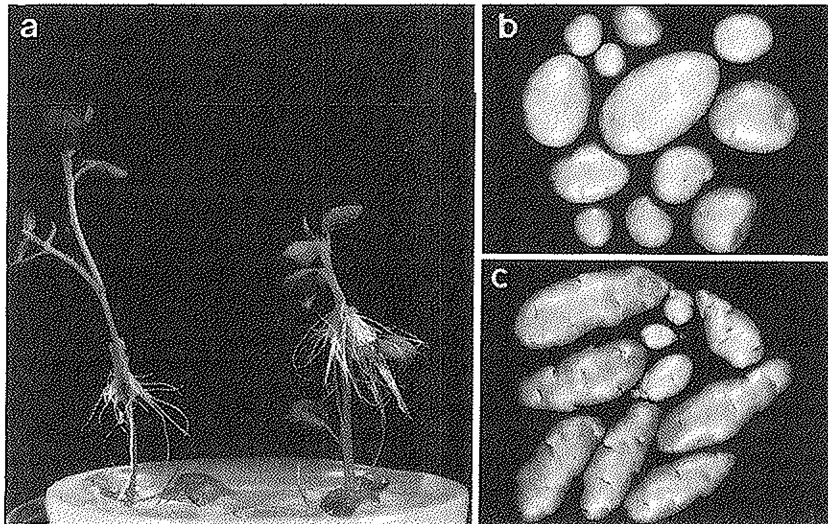

Figure 10. Transformation of potato by *A. rhizogenes*. (a) 'hairy-root' development on wounded potato shoots grown *in vitro* 3 weeks after infection with a suspension of *A. rhizogenes*. (b) tubers of a normal (untransformed) plant, cv. Desirée. (c) tubers of an *A. rhizogenes* transformed potato plant regenerated from excised hairy-root tissue.

ment for tuberization was altered and early tuber formation was very pronounced. Desirée plants containing T-DNA from *A. rhizogenes* developed an extensive root system and produced tubers with prominent and abundant eyes (*Figure 10*).

These results demonstrated the principle that transformed potato plants can be derived from commercial cultivars. In addition, the plants are of scientific interest. They show that the introduction of known growth-modifying genes resulted in specific changes in growth and development of the potato plant, as well as in tuber production. The transformed phenotype was maintained over successive years of field evaluation, indicating stability of expression of the transformed genes (Ooms *et al.*, 1986a). These results illustrate the potential of transformation for studying the pleiotropic effects of specific genes on growth and development of the potato plant. In more recent experiments it has been demonstrated that transformed plants which are normal in phenotype except for the expression of a single gene product, such as the antibiotic resistance mentioned earlier, can be isolated (An, Watson and Chiang, 1986; Ooms *et al.*, 1987).

Transformation in potato breeding

The demonstration that antibiotic-resistant transformed potato plants that are otherwise phenotypically normal can be isolated, illustrates that the technology is already available for introduction of agriculturally desired genes into potatoes. This raises the issue as to which genes should be introduced.

Several constructions have already been made, based on genes of potential use in plant breeding such as chimaeric genes giving enhanced resistance to herbicides, genes that delay induction of virus symptoms, and the toxin gene of *Bacillus thuringiensis* which confers resistance to certain *Lepidoptera* insects (for review *see* Chapter 3 in this volume). Considerable effort is being placed into isolation and manipulation (and even design) of DNA sequences that, upon introduction into plants, will confer useful attributes.

Conclusions

Potato is amenable to a variety of tissue culture techniques and this promises new opportunities for crop improvement from three approaches. The first is the direct incorporation of tissue culture steps in breeding programmes to improve efficiency. This has been demonstrated by the applications of meristem-tip culture for virus elimination and micropropagation for multiplication of useful stocks and to some extent by the production of haploids by anther culture.

A second possibility is to design new breeding strategies, exploiting the opportunities provided by tissue culture. For instance, the multiplication stages for production of clones could be achieved by micropropagation, rather than tuber generations in the field. In addition, manipulation of ploidy by chromosome doubling in callus makes breeding using monohaploids and dihaploids more attractive. The existence of somaclonal variation also offers a

new breeding strategy. Variation has been demonstrated among regenerated plants in useful characters and somaclones tested in field trials over several generations. However, in this instance, it will be necessary to understand the basis of the variability and to demonstrate stability in the field before the potential of somaclonal variation can be fully realized.

Finally, tissue culture also provides an unique experimental approach in which three main areas are developing—mutant selection, protoplast fusion and transformation. Of these, protoplast fusion and transformation are the most promising. Protoplast fusion provides a means of producing hybrids (both inter- and intraspecific) which cannot be derived sexually and should improve the exploitation of the wide gene pool in potato. Furthermore, by genome fragmentation of one of the donor protoplasts, partial transfer of genetic traits should be possible. This will be particularly useful for introgression of characters, the genetic basis of which is unknown or complex. Problems which are now being tackled include the extension of present methods to fusion partners of interest to breeders, which may not carry useful markers for selection of somatic hybrids, and further study of the stability and fertility of the resultant hybrids.

Transformation provides a precise and direct means of transferring genetic information. Experimental data suggest that the methodology is applicable to a wide range of potato cultivars, thereby providing a universal method of obtaining novel potato plants differing from their parental cultivar in a specific phenotypic way, as a result of the introduction of a specific gene. Transformed plants of established potato cultivars have already been produced and could be seen as the first genetically engineered food plants in the world. The speed of development of the technology, linked to rapid *in vitro* micropropagation of transformed plants, may contribute to potato breeding much faster than might be expected for an ordinary improvement in a classical breeding scheme. Practical advances will undoubtedly stem from transformation studies; in addition, such studies will also lead to a better understanding of gene expression in relation to the biochemistry, physiology, growth and development of the potato plant.

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