Successful Application of Biotechnology in Potato

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Abstract: Research on in vitro culture of potato with a view to obtaining disease free material was initiated in 1980. Science then there has been tremendous progress in this area, to the extent that complete plants have been regenerated from isolated protoplasts, cells and pollen. Production of novel plants such as Pombato and Topato were obtained through somatic hybridization between potato and tomato. Micro propagation has made available disease free healthy stocks. In fact, potato is one of the first major crops where biotechnological approaches have been successfully employed for mass production. Biotechnology has literally moved the potato from the test-tube to the field.

Introduction
Potato is the first major food crop where biotechnology has been successfully applied (Bajaj 1987). The in vitro produced disease free plants, somaclones, haploid and somatic hybrids, plants resistant to blight, viruses, nematodes, herbicides and the microtubers produced in test tubes have been moved from the laboratory to the field and propagated on a large scale in various countries (Bajaj & Sopory 1986). The in vitro technology combined with traditional practices have enabled the commercial production of disease free stocks. Efforts are being made to refine methods for the long-term storage of germplasm. Genetic manipulations through cell culture and DNA recombinant technology are underway to improve the nutritional quality, disease resistance and to increase the energy yield of potato.

APPLICATION OF BIOTECHNOLOGY IN POTATO
The range of sophisticated techniques available in potato biotechnology is clearly a broad one. Some of these techniques already having a direct and significant impact on improvement of potato production are reviewed under following heads.

a) Virus free potatoes through meristem culture: Meristem tip culture either alone or in combination with chemotherapy or thermo therapy, has been used to eradicate one or more viruses from more than 100 potato cultivars. Those eliminated include PVA, PVG, PVM, PVRS, PVX, PYY, leaf roll virus, parankinkle virus and potato spindle viroid (Bajaj & Dionne 1986). This technique has become extremely important for potato as a major means of freeing cultivars of virus infection and increasing productivity. Although early workers considered the apical meristem to be free of viruses (Morel & Martin 1952), more recent evidence demonstrated that some viruses are present in this tissue. Heat treatment and chemotherapy of plants prior to meristem tip culture or treatment of meristems during culture have been successful in eradicating a number of viruses that are difficult to eliminate by meristem tip culture alone (Wang & Hu 1980). The elimination of PVX, PVY and PVM from several potato clones by culturing meristems on medium containing virazole was reported (Bajaj 1985). Thermotherapy for virus eradication involves holding the diseased plant at temperatures at or near 37°C for several weeks, long enough to inactivate the virus.

The culture of meristems from potato shoots and tuber sprouts has long history and have several very important applications to potato production. Meristem culture was the first biotechnological approach successfully adopted and applied to obtain virus free potato stocks (Mellor & Stace-Smith 1977). This is based on the observation that the extreme tip of a shoot (Morel & Martin 1952) and a root (Bajaj and Dionne 1965) is free of viruses. Early attempts to culture potato meristems with limited success, although in most cases a few plantlets were produced (Morel and Martin 1955). Growth and development of excised buds was improved by increasing the concentration of inorganic nutrients in the culture medium and adding GA3 Murashinge and Skoog (MS) medium has subsequently proven to the culture of medium of choice. The medium is normally supplemented with GA, although low concentrations of other growth regulators, eg. KIN, BA, and IAA may be beneficial to shoot growth or multiple
shoot formation (Novak et al. 1980). High concentrations of NAA are inhibitory to shoot development and usually induce callus formation. However, low concentrations of the auxin (<0.04 μM) in combination with BA or GA promote the development of vigorous rooted shoots.

The meristem derived virus free plants are cut into small segments with nodes intact (including a leaf and an axillary bud), cultured. The rate of growth and multiplication is faster in liquid media as compared to the agar medium (Schild et al. 1984). The cuttings can be induced to root, planted in the field and multiplied. This method is being used for large-scale tuber seed productions (Foito 1984). Zhang et al. (1993) reported that 40% yield increase in potato using virus free tuber seeds. The application of meristem culture to eliminate virus infection in clonal plants and large scale production in potato have been discussed in many different publications (Nak and Widholm 1993, Djurdjina et al. 1997, Edris et al. 1996, Islam and Chowdury 1998, Struik and Wiersema 1999, Zaman et al. 2001, Ahsan et al. 2003).

a) In vitro tuberization:
The production of microtubers is another approach, which can revolutionize the potato seed industry. The microtubers weigh between 3.5 mg each with two or more eyes. They have the advantage over the test-tube plants in that they can be produced in large numbers irrespective of the season, can be easily stored, packed and shipped with less injury, moreover, their transfer to fresh medium is not required, (Schild et al. 1984). The export of microtubers is already being practiced (CIP 2000). Although in vitro production of potato tubers or microtuberization was achieved more than 40 years ago, the application of microtubers in reliable model research systems has been slow to develop. Several factors such as the use of growth regulators in microtuber induction and growth media, the mitotropism nature of the in vitro system, and cultivar-specific responses have led to interpretive difficulties (Coleman et al. 2001). Shoot culture and tuberization are mainly dependent on pre-existing axillary buds. Shoot elongation occurs on a simple medium while for tuberization an inductive medium, high in sucrose, kinetin and growth retardants, is required (Chandra et al. 1988, Alchanatis et al. 1994). High sucrose levels are necessary for optimal microtuber production (Yu et al. 2000). CIP have developed a rapid, cost effective method that have involved the addition of BAP, CCC and sucrose to the liquid medium used for propagation. BAP or CCC plus BAP and different concentration of sucrose led to the pushing of the potato plantlets to produce microtubers (Edris et al. 1996, Haque et al. 1996, Hossain & Sultana 1998).

c) Haploid breeding: Unlike many Solanaceous species, the cultivated potato has not responded well to anther culture. Despite the difficulties that have been encountered, some progress has been made in the anther culture of cultivated potato. The commercial potato (Solanum tuberosum L.) is essentially an autotetraploid (2n = 4x = 48). The importance of the reduction of the teratoplast potato to the diploid level (with 24 chromosomes) for breeding better clones was first emphasized by Chase (1963). Several factors have emerged that are of critical importance for haploid production in potato, the components of the culture medium, the stage of the development of the microspore and the genotype of the donor plant. Sopory (1978) reported that the addition of 0.18 M (5%) sucrose, 0.5% activated charcoal, 6 μM IAA and 4.0 μM BA to MS medium resulted in a high frequency (40%) of anthers that produced embryos in a selected diploid clone. The high concentration of sucrose was only necessary for the initial stages of embryogenesis. The effect of BA could not be duplicated by other cytokinins and IAA proved to be the most effective hormone. The beneficial effects of activated charcoal on the induction of embryogenesis from microspores during anther culture are not understood, but it has been suggested that it may absorb inhibitors in the medium released from the anther wall.

The development stage of the microspores has a significant influence on the development of embryos (Sopory et al. 1978). In most plant species, including potato, the culture of anthers at an uninucleate pollen stage has given the best response. The frequency of embryogenesis is very low (<4%) in anthers containing tetrads or binucleate microspores. The optimum stage is generally considered to be uninucleate stage.

It is difficult to give convincing reasons as to why uninucleate pollen is the most responsive. It could be that it has not reached the last stage of differentiations i.e. binucleate stage with starch, and hence would be metabolically quite active.

The genotype of the donor plant has the greatest influence of the success of anther culture in potato. It has long been noted that some potato cultivars and dihaploid breeding lines respond more readily in anther culture than others (Wenzel & Uhrig 1981). The responsive of certain genotypes appears to be a heritable characteristic but the mode of inheritance has not been ascertained. The failure of some genotypes to produce viable embryos in culture may be the result of the presence of genes that are lethal in monohaploids or in the homozygous state in diploids, but not in the heterozygous dihaploid donor plant (Wengel 1980). Furthermore, other factors, such as physiological state of the parent and pretreatments to the anthers are effective. In order to achieve success, an optimal interaction has to be achieved between these factors. Using the optimal medium, Sopory and Tan (1979)
directly obtained embryoids in a dihaploid clone H226, H260, H030, H2701 and other hybrid clones.

The production of haploids in large numbers enables the construction of homozygous pure lines after diploidization but by conventional method it takes 6-8 years, and new combinations of characters can be selected in F1. The haploid tissue is also a good source of inducing desirable mutants.

d) Protoplast and somatic hybridization:
Protoplast isolation, culture and plantlet regeneration have been achieved in numerous tetraploid cultivars (Bokelman and Roest 1983) and diploid breeding lines (Wenzel et al. 1979) of S. tuberosum spp. tuberosum, as well as two wild species of Solanum. Shepard and Tolten (1977) were first to report plantlet regeneration from protoplast of cv. Russet Burbank. Since then many workers have refined the original protocols and achieved plant regeneration from protoplast of number of potato cultivars. These developments have been extended by the recovery of somatic hybrids of S. tuberosum and S. brevifrons by protoplast fusion and the transfer of resistance of potato leaf roll virus from S. brevifrons into S. tuberosum by somatic fusion.

These are many protocols for the isolation and purification of protoplast from potato plants and successful culture of potato protoplast has been reported from several laboratories (Bajaj 1987).

So far potato protoplasts have been used as fusion partner of tomato S. chacoense, S. nigrum, S. brevifrons, S. pinatiscium, S. bulbocastanum and S. cardiphyllum by means of conventional chemical fusion techniques (Bajaj 1987). At the same time process of electro fusion has been used to generate fusion products. The electro filed manipulation of aggregation and fusion have been used to analyze the fusion characteristics of protoplast and to generate large numbers of heterokaryons of S. tuberosum which are difficult to fuse by chemical means (Zimmermann 1982). Mclchers et al. (1978) employed protoplast fusion between the sexually incompatible cross potato and tomato, with a view to obtain hybrids. The somatic hybrids thus obtained were termed “potato” or “topato” if bearing potato or tomato plastomes respectively (Michers 1980). Plastid DNA restriction analysis confirmed these results by failing to detect in the hybrids any recombinant plastid DNA types (Schiller et al. 1982). Though the original intention to produce a hybrid plant which would bear tomato fruits and potato tubers has not met with success, however, its possibility is hot yet ruled out. CIP has developed a protoplast fusion program in potato. This technique has proved useful in the transfer of cytoplasmic characters, particularly cytoplasmic male sterility in tobacco. (Aviv et al. 1980).

e) Somaclonal variation: Morphological variation has often been observed in potato plants regenerated by in vitro techniques involving an intermediate callus phase. Variation in a number of horticultural traits and disease resistance has been reported among plants regenerated from potato protoplast (Thomas et al. 1982). With the exception of sugarcane, no world crop rivals with potato as a model for improvement via somaclonal variation (Orton 1984). The possible in vitro selection for tolerance to toxic agents, including toxins from pathogens, increased the interest to use tissue culture techniques for the application of reliable selection pressure in order to screen up crop plants for disease resistance, herbicide tolerance and stress tolerance (Lepovier et al. 1985).

Van Herten et al. (1981) observed variation frequencies from 50.3% and 12.3% among plants regenerated from potato petiole and leaf calli respectively, in the absence of mutagenesis treatments, some of these variations included growth habit, maturity date, tuber characteristics and light requirements for flowering. Five hundred protoclonies of Russet Burbank were treated with culture filtrates of A. solani (causal agent of early blight) and 800 with P. infestans (causal agent of late blight). Two to three percent plantlets derived from treated protoclonies were found resistant to these pathogens (Meulemans et al. 1987). Resistant to some other fungi (Matern & Strobel 1987) and several herbicides tolerant potato plants were also obtained (Weller et al. 1987). Somaclonal variation with useful properties have been produced in potato and such variants are resistant to early blight (Alternaria solani) and to multiple races of Phytophthora infestans (De 1992, Chaudhury 1994, Yee et al. 2001, Khatun et al. 2003, Nasrin et al. 2003).

f) In vitro mutation: The genetic variability can be originated from cell and tissue cultures and the phenomenon frequently observed in vitro in several plant species has been extensively discussed by several authors (Thomas et al. 1982). Variation has been reported in plants regenerated form protoplast and in plants derived from in vitro culture of different somatic tissues. Genetic variation can be induced as a consequence of the culture conditions adopted, with particular reference to medium composition, hormonal balance, culture duration etc. Besides, through adventitious shoots, regeneration of plants can be obtained from pre-existing mutated somatic cells that normally do not have any chance of revealing themselves. Rosest and Bokelmann (1980) performed a detailed experiment aimed at analyzing the mutation breeding potential of in vitro adventitious buds in potato. They cultured non-irradiated and irradiated at different doses of X-rays on regeneration medium. The irradiation strongly affected the regeneration capabilities of the explants; the higher the irradiation doses the higher was the inhibition of shoot regeneration.
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In potato the propagation by micro culture has been clearly shown to be a valuable too for the rapid isolation of solid mutants (Van Harten et al. 1981) and a large numbers of mutants were isolated after about a month of transplantation in the open field. All the mutants considered homogeneous confirmed their stability through two further field trials. Thus irradiation (X-ray or gamma ray) and propagation of in vitro grown plants seem to be rapid and easy methods for the induction of mutations can not obviously, replace cross-breeding, nevertheless, it can represent a valuable support to potato breeding programmes.

g) In vitro conservation: In vitro systems for the storage and preservation of potato germplasm have received considerable attention and practical alternatives to conventional means of storage of vegetative material are now available. Cryopreservation and minimal growth storage have been applied to potatoes, with generally satisfactory results (Bajaj 1985). The work on the cryopreservation of potato in liquid nitrogen was initiated by Bajaj 1985 cell cultures and protoplast derived cell colonies. Entire plants capable of undergoing normal tuberization was obtained from cultures freeze preserved for 4 years (Bajaj 1987). This has been accomplished for potato primarily by reducing incubation temperatures and increasing the osmolarity of the culture medium (Roca et al. 1979).

With techniques at hand for long term storage of pathogen free material, international distribution of germplasm should become less restrictive. Aseptic cultures were from CIP distributed to 14 countries (Roca et al. 1979) in accordance with the quarantine regulations. Meristem culture techniques provide adequate safeguards to prevent the dissemination of dangerous pests and diseases into countries or regions. The procedures also permit rapid multiplication of disease free stocks from a few imported cultures; a 5-10 fold increase of plantlets can be achieved every 2-3 weeks.

During the last decade, with the improvement PCR protocols and due to availability of automatic thermal cyclers commercially, the application of PCR have increased manifold. DNA fingerprinting in agriculture is a front line research in biological sciences. Serological process for virus detection in crop plants is costly, time consuming and sometimes failed to detect low copy virus hidden in plant cell. In advanced countries many scientists have been actively engaged to develop PCR based low cost technologies to detect somaclonal variation.

h) Genetic engineering: The insertion of synthetic gene in potato plants has been successful (Dodds 1987). Evidence has also been obtained that the gene is transcribed and produces a corresponding messenger RNA molecule, which is then translated into this plant to produce synthetic protein. On the basis of the success with the synthetic protein gene, CAP has directed attention to the possible use of genetic engineering to give the potato plant resistance to pests diseases (Jayness et al. 1985). There are many reports on the genetic transformation of potato (Sheenan & Bevan 1988, Visser et al. 1989, Vadya & Belknap 1992). Potatoes were one of the first crop plants in which transgenic plants were successfully regenerated. Although potato transformation can be accomplished by direct uptake of DNA into protoplasts (Feher et al. 1991), Agrobacterium-mediated transformation using binary vectors is the preferred method and is performed routinely in many laboratories (Conner et al. 1991). The importance of potato transformation is increasing as a means of introducing better and useful traits into many cultivars of economical relevance for integrated crop management (Sarker and Mustafa 2000). Some of the main traits improved by genetic engineering are virus resistance viz. PVX, PLRV, PVS, PVY, broad spectrum; bacterial resistance viz. soft rot, wilt; insect resistance viz. potato tuber moth, tobacco hornworm, Colorado potato beetle; herbicide resistance viz. glufosinate and bialophos, chloroauron; tuber quality traits viz. reducing browning, increased starch, highly branched starch, amylose-free starch, human serum albumin, cyclodextrin production (Tacke et al. 1996, Jeong et al. 2001, Ahsan 2003).

Conclusion
For many years biotechnology has been applied to improve potato production by means of micropropagation, pathogen elimination and germplasm conservation. However, some of these techniques are still being refined and improved. Intermediate level technologies such as in vitro tuberisation, and embryo and anther culture are having some direct application on germplasm distribution and germplasm improvement. The most sophisticated technologies such as genetic engineering and protoplast fusion have enormous potential to improve potato production but care must be exercised in the translation of that potential into reality.

It is important to see biotechnology not as a scientific discipline but rather as a range of techniques. These techniques are of differing degree of complexity forming a complete spectrum of technologies, for agricultural application the most important feature is the integration of these techniques to improve potato production in its widest possible sense.

References
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