Food applications of plant cell cultures

G. A. Ravishankar and L. V. Venkataraman
Central Food Technological Research Institute, Mysore 570 013, India

Plant tissue and cell culture techniques have been applied to modify the crop plants at preharvest and postharvest levels for increased productivity, enhanced quality, easy agronomic management and for improved characteristics of processed foods. Improvement in nutritional qualities and disease elimination, disease resistance, tolerance to environmental conditions, pesticides and herbicide, and mass propagation of crop plants where application have been realized is presented. Status of production of food value phytochemicals has been reviewed. Future directions of food application of cell culture techniques have also been projected.

PLANT tissue and cell culture (PTCC) techniques have now been realized for its potential in agriculture and industry due to the ability of plant cells to express morphological and chemical totipotency. PTCC comprises various techniques with a range of application. These techniques have been applied for genetic improvement of plants for higher yield, disease elimination and disease resistance. The importance of its role in micropropagation of ornamental plants, food crops and tree species has already been realized. Improvement of agricultural cultivars with specific qualities in food materials by PTCC methods is fast becoming an important area of biotechnology.

This paper gives an overview on the use of the above PTCC in the improvement of quality and quantity of food materials derived from conventional and nonconventional sources of plants. Table 1 summarizes these applications based on the current knowledge.

Preharvest aspects

Improvement in nutritive value

Increased production of deficient amino acid, viz. lysine in cereals, threonine in wheat and rice, and tryptophan in corn is possible by PTCC methods. Halsall *et al.*[1] and Brock *et al.*[2] proposed a scheme for development of lysine over producing cereals. Cells insensitive to feedback inhibition of lysine and threonine[3, 4] or cells tolerant to S-aminoethyl cysteine (S-AEC) have given rise to lysine and threonine over producing mutants. S-AEC acts as an analogue of lysine and is useful in development of lysine over producing mutants of barley[5]. Selection of amino acid over producing cell lines has been reported in more than 12 plant species[6] of which successful ones are shown in Table 2.

The work of Hibberd *et al.*[4] has demonstrated that the regenerated *Zea mays* plants from methionine, lysine and isoleucine over producing cell lines, showed increased free amino acids in the kernel. Moreover, this character was heritable thus demonstrating a highly useful potential of PTCC for cereal crop improvement. Increase in sugarcane yield (32%), sucrose yield (34%), and stalk number (6%), over the donor was obtained in somaclones. Llewellyn *et al.*[7] demonstrated that phaseoline gene could be incorporated into tobacco genome and expressed in seeds. This has opened up the possibility of increasing nutritional value of edible seeds.

Reduction/elimination of antinutritional factors

Many cereals, pulses and oilseeds contain a range of antinutritional factors that affect their full utilization. The nutritional quality of food depends on the amount of nutrients present in the food consumed as well as the levels of antinutritional factors. Trypsin inhibitors present in large amounts in legumes[8], leafy vegetables, cereals[9] can affect enzymic digestion. Chlorogenic acid in sunflower seed meal can inhibit the activity of digestive enzymes. Solanine, an alkaloid of potato[10], amylase inhibitor[11], thioglycerides in rapeseeds which act as goitrogenic stress factors[12], are the other most common antinutritional factors[13]. Phytic acid in plants chelate ions like calcium, magnesium, iron and zinc making them insoluble. Gossypol of cotton meal is known to chelate metals and can cause anaemia in nonruminants. Tannins present in legumes, cereals and

Table 1. Plant tissue and cell culture for food application.

Applications	Techniques*
Preharvest	
Improvement in nutritive value	1,2,3,12
Reduction and elimination of antinutritional factors	1,2,3,12
Disease elimination/resistance	3,4.11,12
Herbicide, pesticide, drought, salt, frost tolerance	1,2,3,12
Increased yields	3,6,7,8,12,13,14
Mass propagation	1,2,4,5,12
Postharvest	
Plant cell cultures for flavours, colours, sweeteners	9,10,11,13,14
Novel compounds/processes/products	3,5,7,9,10,11
Easy harvesting	3,12
Fruits and vegetables which withstand long transportation	3,11
Improved and efficient processing	2,3,9,10,13

*Techniques: 1, Callus cultures; 2, cell cultures; 3, somaclonal variations; 4, meristem culture; 5, embryo culture; 6, anther culture; 7, protoplast culture; 8, *in vitro* fertilization; 9, immobilized cell culture; 10, bioreactor processes; 11, plating techniques; 12, propagation methods; 13, genetic engineering; 14, mutation.

Table 2. Regeneration of plants from mutant cell lines using amino acid and their analogues.

Selective agent	Plant*	Mutagen
S-amino-ethyl-cysteine	*Arabidopsis thaliana*	Ethylmethane sulphonate
	Hordeum vulgare	Azide
	Oryza sativa	—
Hydroxy proline	*Hordeum vulgare*	Azide
Lysine plus threonine	*Zea mays*	With or without azide

*All showed plant regeneration.

vegetables inactivate enzymes and make proteins indigestible and reduce bioavailability of iron and B12 vitamins[12]. Nothing specific is known about the flatulence factors in beans. They are however, under genetic control and there exists a possibility to reduce or eliminate them by genetic selection. Nutritional stress factors present in the foods are given in Table 3.

In India lathyrism is prevalent in Madhya Pradesh, Bihar, and Uttar Pradesh, where *Lathyrus sativus* (kesar dhal) is used as a part of food by economically weaker sections. This contains a toxic amino acid which leads to crippling in humans. In spite of governmental efforts to ban cultivation of *Lathyrus* it has not been effective to control the same since the poor farmers find this crop easy to cultivate under drought condition. Hence there is an urgent need to obtain toxin-free *L. sativus*.

Applicability of somaclonal variation by elimination of nutritional stress factors has been achieved recently[14]. First application of this technique was shown in *Amaranthus gangeticus* a leafy vegetable, wherein reduction in oxalates was obtained. This was done by using a iron-ferron dye 7-iodo-3-hydroxyquinoline 5-sulphonic acid as an indicator of oxalate in cell clones. Similar method can be adopted for spinach, knolkol, tomato, etc. which are also rich in oxalates. Plating method has been applied to reduce tannins in sorghum, trypsin inhibitor in maize, barley, rye, etc., where inhibitor is not heat-labile[15]. Technique of antisense RNA can be used to eliminate antinutritional factors by blocking any one step of biosynthetic pathway. Tomato plants with reduced polygalacturonase activity have been developed by Calgene USA by antisense RNA technique to decrease fruit rotting, to increase solid content and viscosity[16].

Modification of agronomic traits

Somaclonal variation is advantageous over traditional methods of breeding and selection, as cell cultures can be exposed to selective conditions of chemical and physical nature and variant cells can be regenerated to plants. This system offers a method of screening billions of potential cells in a single flask which is otherwise difficult by traditional procedures. The regenerants have shown great variability in characters which are also heritable[17]. While technique of somaclonal variation has been widely used to improve agronomic qualities such as salt, frost, herbicide tolerance, drought and disease resistance, its application to improve food qualities is being attempted in the past few years only. Few recent examples of application of somaclonal variation are as follows[14].

Increased seedling vigour of lettuce; improved rice protein content; *Fusarium* resistance in alfalfa; increased solid content in tomato; *Pseudomonas* and *Alternaria* resistance in tobacco; early flowering in soybean, increase in sugarcane yield.[18]

Salt-resistant cell lines and plants have been obtained by PTCC methods in crop plants like tobacco (*Nicotiana tabacum*)[19], flax (*Linum usitatissimum*)[15], wheat (*Triticum aestivum*)[20], rice (*Oryza sativa*)[21]. The heritability of salt tolerance has been shown in *Oryza sativa*[22]. Oats (*Avena sativa*)[23] and pearl millet (*Pennisetum americanum*)[24] and these have been regenerated from callus cultures. Use of proline analogues for increasing resistance to salt has been reported[25].

Plants for resistance against herbicide, chilling, freezing, drought have been selected[26,27]. With the advent of genetic engineering, the gene for toxic protein production from *Bacillus thuringiensis* has been transferred to tobacco and tomato plants and expression of gene has been achieved. Such genetically engineered plants have proved resistance to several insect pests[28,29].

Demonstration of agronomically significant transformations involving herbicide tolerance by recombinant DNA technique is important. The herbicide *N*-phosphonomethyl glycine or glyphosphate inhibits aromatic biosynthetic pathway at its sixth step—namely, enolpyruvylshikimate-3-phosphate (EPSP) synthase which is involved in biosynthesis of essential amino

Table 3. Nutritional stress factors in food plants.

Food	Inhibitor	Nature	Action
Cottonseed meal	Gossypol	Polyphenol	Decreased food intake
Castorbean	Toxalbumin	Protein	Paralyses/respiratory/vasomotor
Cereals	Phytin	Inositol hexaphosphate	Makes calcium unavailable
Spinach, knolkol, tomato	Oxalic acid	Organic acid	Makes calcium unavailable
Lima beans	Antitrypsin	Protein	Inactivates trypsin
Corn	—	—	Makes nicotinic acid unavailable
Lathyrus sativus	Toxic amino acid	amino acid	Neurotoxin

Modified after Teutonico and Knorr[14].

acid—phenylalanine, tyrosine and tryptophan. It has been indicated that over production of a bacterial EPSP synthase in bacteria resulted in herbicide tolerance. The EPSP synthase cDNA of petunia was flanked by the cauliflower mosaic 35 S 5′ promoter and 40 S 3′ regulatory regions. Petunia cells with this construct resulted in herbicide resistance and over production of EPSP synthase by 30–60 folds. The plants regenerated from above cell lines were found tolerant to 0.89 kg/ha herbicide. Control plants were killed at 0.22 kg/ha with herbicide. Similar strategies are being used to create plants tolerant to several other herbicides such as atrazine, imidozolidinone series, sulphonylurea and phosphonitricine[30]. Transfer of atrazine tolerance from *Brassica campestris* to *B. napus* (canola) was done at the University of Guelph. The new atrazine-tolerant canola was approved by Agriculture Canada for plantation in 20–30,000 acres[31].

Disease elimination/resistance

Generally viruses are passed on from one generation to other by vegetative means and entire population of plant can be infected by viruses, with decreased yields. Meristematic regions of shoot and root apices have been shown to be virus-free or contain low concentration of viruses[32]. By culturing the meristem the disease-free plants can be raised. This technique is also useful for elimination of virus which are seed-borne. About 10% of viruses are propagated through seeds. In tomato, TMV virus is localized in seed coat. In legumes, virus is carried in the seeds. Seed-borne mosaic virus in pea was eliminated successfully in over 100 breeding lines[33]. Sugarcane crop is vulnerable to several viruses and fungi[34]. These plants, free from leaf hopper-transmitted Fiji virus, were obtained from meristem culture. Disease-resistant plants for Fiji disease and downy mildew (*Sclerospora sacchari*) were also obtained[35, 36]. About 56 plants were made virus-free by meristem culture by 1983[37]. The indexing of viruses and viroids using DNA probes and or monoclonal antibodies is being developed for detecting extremely low levels of these pathogens[38]. Currently used DNA probes require ^{32}P labelled isotopes and detection by X-ray film radiography. Attempts are being made for devising nonradioactive test, ELISA, for indexing. Labelled probes are used presently in Philippines for stunt on rice disease of Aisa and spindle viroid disease of potato at Peru. Recombinant DNA approach of expression of viral coat protein gene in tomato and potato has successfully demonstrated the protection against the viral infection[39, 40]. Expression of antisense RNAs of CMV virus in tobacco is being studied for virus resistance[41]. This opens up a possibility of genetically engineered protection in agriculture. Induction of disease resistance is another promising feature of plant tissue culture. The toxins isolated from pathogens such as *Alternaria* have been used as stress factor to produce toxin-resistant clone of potato which eventually confers resistance to pathogens[42]. Induction of disease resistance in plants by chitinase gene expression is being explored[43].

Haploids/protoplasts

Since the first report[44] of *in vitro* haploid induction from anther culture there are over 200 spp of haploid plants produced. This has led to shortening the time required for obtaining homozygous diploid lines. Chinese have developed a high-yielding rice variety by this technique which is presently grown in Eastern province in 100,000 ha. Winter wheat developed by Chinese had exhibited increased grain number, more tillers, resistance to stipe rust and powdery mildew and with short stem.

It is now possible to microinject pollens (or intact cells) with desired genes and cross-pollinate to produce improved plants[45]. Protoplast fusion method has not made significant impact on plant improvement except in the areas of cybrids. Cytoplasmic inheritance such as herbicide/pesticide tolerance, male sterility, toxin resistance, etc. have been expressed in cybrids. Microinjection and electroporation techniques have widened the application of protoplast for genetic manipulation[46].

Mass propagation

The *in vitro* potato tubers are small and easy to transport compared to normal tubers. They can be raised in laboratory conditions and conveniently used as propagule. Generally, one fifth of potato produced is reused in cultivation. By adopting *in vitro* mass tuberization, the availability of potato for human consumption increases besides easy transportation. Already these techniques are used in Peru and Vietnam[37]. Potato production in Vietnam by disease-free stocks has increased yield from 10 tonnes/ha to 18 tonnes/ha[47]. Production of microtubers of *Dioscorea alata* an edible Yam has been reported[48]. Another interesting possibility is a somatic hybrid of *D. alata* (edible underground tuber) and *D. bulbifera* (aerial tuber) that would yield delicious aerial tubers which can easily be harvested[48]. Microtubers of *D. rotundata* have been recently developed for propagation and to facilitate international exchange[49]. Several crop plants are presently propagated for cultivation purpose by tissue culture methods. Oil palm clones are expected to yield 30% more oil than mixed seed population[50].

In vitro somatic embryogenesis has been reported in over 100 plant species[51] including major cereals—rice and wheat[52]. Several delivery systems have been developed and commercialized based on synthetic seed technology of encapsulation of somatic embryos[53].

Production of phytochemicals (metabolites)

Plant cells cultured *in vitro* produce wide range of primary and secondary metabolites of economic value. The advantages of this technology over the conventional agricultural productions are as follows:

They can be grown in controlled conditions free from agro-climatic constraints.
Uniform production throughout the year is ensured independent of seasonal variations.
Rapidity of production.

Production of phytochemicals from plant cell cultures has been presently been on pharmaceutical products. It is now realized that this technology can be used for production of food additives, biopesticides, etc. Synthetic food additives and colourants are being banned due to their toxicity. Synthetic pesticides used for storage of foodgrains are harmful to environment and humans. Under such circumstances plant cell cultures hold promise for production of these natural compounds. Production of food colours, flavours, sweeteners and pesticides is reported from plant cell cultures.

Food colours

The total world market for natural and synthetic pigments is estimated to be $ 150–200 millions[54]. The selected cell lines of different plants have been used for pigment production, e.g. *Lithospermum erythrorhizon*[55], *Daucus carota*[56], *Prunus persica*[57], *Haplopappus gracilis*[58], *Vitis vinifera*[59], *Catharanthus roseus*, *Euphorbia millii*[60].

Employing two stage culture system anthocyanin at 13% dry weight has been achieved[61] with an yield of 830 mg/l/15 days. Anthocyanin from conventional sources is expensive and cost $1,250 to 2000/kg. Canadian and Israel biotechnology firms have now ventured into commercial exploitation of anthocyanin by plant cell culture. A rare anthocyanin was isolated from mutant cells of *Daucus carota*[62] with a yield of 300 mg of purified anthocyanin per litre of cell suspension. The special feature of this anthocyanin was its 2–3 times the normal colour which is stable at acidic pH (2–5) for 6 months. Shikonin (red pigment) production from cell culture has been commercialized by Mitsui Petrochemical Ltd, Japan. The advantage of this technology is high production capacity at 12–20% of dry weight of cells compared to intact plants containing 1–2% Shikonin in its roots. Currently Shikonin is sold at $ 4000/kg and is economically significant for cell culture production.

Food flavours

Flavour in a plant is generally a mixture of compounds in definite proportions. According to one estimate[61], 4300 different flavour compounds have been identified in foods. Certain flavours consist of one or few related compounds, viz. 2-isobutylthiazole (tomato flavour), methyl-ethyl cinnamates (strawberry), methyl anthranilate (grape), benzaldehyde (cherry), menthol (mint), safranal (saffron)[61].

There are some studies aimed at production of flavour compounds by plant cell cultures. Carew and Staba[63] suggested that essential oil cannot be produced by tissue cultures since they lack oil glands. There are many reports which support the view that at least some level of morphological differentiation is needed to get flavour production in cultures, e.g. celery[64], onion[65]. Partially differentiated callus or suspension cultures of *Ruta graveolens*, chamomile, perilla, coriandrum, pepper mint[66] show synthesis of flavour compounds. Capsaicinoids are produced in immobilized cell cultures of *Capsicum frutescens* without organ differentiation[67]. Townsley[68] found that cocoa aroma can be produced by senescent cells.

The flavours of onion and garlic are also produced in tissue cultures. Three flavour precursors, *S*-methyl and *S*-propyl-l-cysteine sulphoxides and *trans*-prop-l-enyl-l-cysteine sulphoxide are hydrolysed by alinase enzyme when tissue is cut to give volatile flavour components[66]. Quantity of chlorophyll has been used as marker for flavour production in celery cell and tissue cultures[66] and for selection of high-yielding cell lines. Callus cultures of saffron have been established which produce flavour and pigments[69]. Expensive fragrances and flavours like jasmine roseoxide, nootkatone and valencene can also be considered for production by tissue culture. Nootkatone is a potent flavouring agent with fruity odour obtained from grape tissue and valencene possesses orange flavour[70].

Sweeteners and food additives

There is need to find nonnutritive and safe sweeteners for various food and pharmaceutical applications. This has led to the discovery of a series of dihydrochalcones developed by USDA investigators which can be derived from flavone glycosides that occur in citrus peels[71]. The important nonnutritive sweeteners are miraculin (Miralin) from fruits of *Synsepalum dulcificum*[72], stevioside from *Stevia rebaudiana*[73], thaumatin (Monellin) from *Thaumatococcus danielli*[74], 8,9-epoxy-perillaritine from *Perilla nankinesis*[75], and the hydrofluorene diterpenoids from pine tree rosin[76]. The scarcity of many of these plant species and problems involved in adapting them have increased the need to develop tissue culture systems for production of flavours. *Stevia* cultures have been established to study steviol biosynthesis[77], aglycone steviol can be biotransformed in *Stevia rebaudiana* and *Digitalis purpurea*[78] to obtain glycosides (Steviolbioside and Stevioside) which are sweet. Recombinant DNA techniques have been used to transfer thaumatin gene from its parent plant to yeast

system[79]. The genetically altered organism produces thaumatin which is 3000 times sweeter than sucrose. Stevioside and thaumatin can be produced in large scale as an alternative to synthetic sweeteners[70].

Capsaicin, a pungent food additive[80], is one of the widely investigated food compounds for production by cell cultures[81]. It is produced in fruits of *Capsicum* sps. The cell cultures of *Capsicum frutescens* have been immobilized and its capsaicin production has been enhanced by precursor biotransformation[81]. The immobilized cells release capsaicin to medium, thereby obviating harvest of cells[82]. Nutritional stress has been known to increase capsaicin production in *Capsicum annuum* cells by over 10-folds[82]. Saffron tissue cultures with flavour and colour principles have been developed[83,84].

Other products

Biotin over producing cell lines of *Lavendula vera* have been developed[85]. Safflower cell cultures with high vitamin E activity among tocopherol analogues have been produced. Several biosynthetic precursors such as phytols, geranyl geraniol, shikimic acid and homogentisic acid were administered to increase tocopherol(s) production. *Cynara cardunculus* known for its production of phenol and flavonoid derivatives used widely for milk clotting and cheese production has been obtained by cell cultures[86].

Production of pyrethrins, a bioinsecticide by cell cultures of pyrethrum (*Chrysanthemum cinerariaefolium*) has been reported[87]. Cell culture-derived pyrethrins have been shown to exhibit potent insecticidal activity on insects infesting stored foodgrains. High-yielding cell lines of pyrethrum can be obtained from high-yielding donor plants[87]. Further work is needed for using these high-yielding cell lines in commercial production.

Novel compounds/processes/products

Application of protoplast fusion for development of hybrids and cybrids, somaclonal variation and genetic engineering will bring dramatic results. There is need to develop fruits and vegetables which can withstand transportation to long distances. This can be attempted by screening somaclonal variants. The novel methods of improving the quality of foods by altering their biochemical nature can be attempted. For example, attempts are being made for manipulation of carbohydrate metabolism[88] by recombinant DNA technology which when applied to cereals can improve texture, cooking properties of rice, enhanced sweetness and mouth feel of corns, improved staling characteristics of wheat flour for baked foods[89]. Processed wheat products such as *chapati*, bread or noodles require different dough characteristics that are imparted by specific gluten content in grains. Similarly, control of starches with varying degrees of branching and chain length to improve texture and storage properties of grains is envisaged by genetic engineering methods[90].

Heinz Co and DNA Plant Technology Laboratory, USA, developed supertomatoes with high solid content by screening somaclones which reduced shipping and processing costs. According to an estimate, in the US alone, for every per cent increase in tomato solids processors would save $ 100 million per year[91].

The attempts presently in progress[92] to manipulate lipid biosynthesis to change triglyceride composition for the production of coconut type oil in soybean and rapeseed will be a novel approach. Newer techniques such as microinjection of foreign DNA, electroporation methods for protoplasts, and improved technique of agrobacterial transformation as applicable to cereals and monocots need to be developed to realize the potentials of interdisciplinary approach to PTCC methods. Development of two stage culture methods as done for pigments[93] should be adopted for important metabolites. Recent trends in use of elicitors for enhanced metabolite synthesis will prove to be a useful tool. Improvement is needed in bioreactor design for scaling up of plant cell cultures. Development of photoautotrophic cell culture system needs to be done to harness the photosynthetic potentials of plant cells. Innovative and imaginative approaches as demonstrated for development of lemon vesicles in tissue culture[94], or production of saffron stigma from ovary culture[95] are needed to give a turn from traditional approaches.

Conclusion

In the Western countries there is already abundant production of food materials. The emphasis in biotechnology in the West is changing from increased productivity of field-grown plant to methods of unconventional production of food *in vitro*. Plants are being modified to facilitate easy processing and to make the products of high quality. The biotechnology in the West is dealing mainly with health and industrial processes. India is basically an agricultural country and there is lot of scope for improvement in various crops to suit our varied environment. Green revolution in 1960s has only brought about increase in cereal output. There is need to apply the biotechnological methods for improvement in oilseeds and pulses. The same can be achieved by obtaining plants tolerant to adverse conditions such as drought, floods, salinity, alkalinity, etc. Coupled to this, several innovative steps mentioned earlier may bring about a quantum jump in our food output in terms of quantity and quality. In future years appropriate technology has to be developed, perfected and practiced in India as it is not always relevant to adopt the technology developed by the Western countries.

1. Halsall, O., Brock, B., Langridge, J. B., *CSIRO Division of Plant Industry, Genetics Report*, 1972, p. 31.
2. Brock, R. D., Friederick, E. A. and Langridge, J. B., in *Nuclear Techniques for Feed Protein Improvement*, International Atomic Energy Agency, Vienna, 1973, p. 329.
3. Gengenbach, B. G., Walter, T. J., Green, C. E. and Hibberd, K. A., *Crop. Sci.*, 1978, **18**, 472.
4. Hibberd, K. A., Walter, T., Green, C. E. and Gengenbach, B. G., *Planta*, 1980, **148**, 183.
5. Bright, S. W. J., Featherstone, L. C. and Miflin, B. J., *Planta*, 1979, **146**, 629.
6. Flick, C. E., in *Handbook of Plant Cell Culture*, vol. 1, *Techniques for propagation and breeding* (eds Evans, D. A., Sharp, W. R., Ammirato, P. V. and Yamada, Y.), Macmillan Publ. Co., New York, 1983, p. 393.
7. Llewellyn, D. J., Dennis, E. S. and Peacock, N. J., in *Molecular Form and Function of the Plant Genome*, Proc. NATO Advanced Studies Institute, Renesse, Netherland Plenum Press.
8. Liener, I. E. and Kakade, M. L., in *Toxic Constituents of Plant Foodstuffs* (eds Liener, I. E.), Academic Press, New York, 1980, p. 7.
9. Chen, I. and Mitchell, H. L., *Phytochemistry*, 1973, **12**, 327.
10. Whitaker, J. R. and Feeney, R. E., in *Toxicants Occurring Naturally in Foods*, National Research Council, National Academy of Sciences, Washington, D. C., 1973, p. 276.
11. Jaffe, W. G. and Vega, C. L., *J. Nutr.*, 1968, **94**, 203.
12. Liener, I. E., in *Toxic Constituents in Plant Foodstuffs* (ed. Liener, I. E.), Academic Press, New York, 1980, p. 283.
13. Hamblin, J. and Kent, S. P., *Nature*, 1973, **245**, 28.
14. Teutonico, R. and Knorr, D., *Food Technol.*, 1984, **38**, 120.
15. McHughen, A. and Swartz, M., *J. Plant Physiol.*, 1984, **117**, 109.
16. Anonymous, *Biotechnology*, 1989, **7**, 337.
17. Larkin, P. J. and Scowcroft, W. R., *Theor. Appl. Genet.*, 1981, **60**, 197.
18. Liu-M. C. and Chen, W-H., *Euphytica*, 1976, 393.
19. Nabors, M. W., Gibbs, S. E., Berstein, C. S. and Meis, M. E., *Z. Pflanzenphysiol.*, 1980, **97**, 13.
20. Maddock, S. W., Lancaster, V. A., Rissiott, R. and Franklin, J., *J. Exp. Bot.*, 1983, **34**, 915.
21. Yoshida, S., Ogawa, M., Suenaga, K. and Chon Ye, H., in *Cell and Tissue Culture Techniques for Cereal Crop Improvement* (eds Hu Han and Vega, M. R.), Science Press, Beijing, China, 1983, p. 237.
22. Yamada, Y., Ogawa, M. and Yano, S., in *Cell and Tissue Culture Techniques for Cereal Crop Improvement* (eds Hu Han and Vega, M. R.), Science Press, Beijing, 1983, p. 229.
23. Heyser, J. W. and Nabors, M. W., *Z. Pflanzenphysiol.*, 1982, **107**, 153.
24. Vasil, V. and Vasil, I. K., *Am. J. Bot.*, 1982, **69**, 1441.
25. Dix, P. J., Mclysaght, U. A. and Plunkett, A., in *Plant Tissue Culture and Its Agricultural Applications* (eds Withers, L. and Alderson, P. G.), Butterworths, London, 1986, p. 469.
26. Hughes, K., in *Handbook of Plant Cell Culture*, vol. 1, *Techniques for Propagation and Breeding* (eds Evans, D. A., Sharp, W. R., Ammirato, P. V. and Yamada, Y.), Macmillan Publ. Co, New York, 1983, p. 442.
27. Tal, M., in *Handbook of Plant Cell Culture*, vol. 1, *Techniques for Propagation and Breeding* (eds Evans, D. A., Sharp, W. R., Ammirato, P. V. and Yamada, Y.), Macmillan Publ. Co, New York, 1983, p. 461.
28. Adang, M. J. *et al.*, in *Proc. VI Int. Congr. Plant Tissue and Cell Culture* (eds Somers, D. A., Gengenbach, B. G., Biesboer, D. D., Hackett, W. P. and Green, C. E.), IAPTC, USA, 1986, p. 404.
29. Fischhoff, D. A. *et al.*, *Biotechnology*, 1987, **5**, 807.
30. Jaworski, *Cereal Foods World*, 1987, **32**, 754.
31. Netzer, W. J., *Biotechnology*, 1984, **2**, 939.
32. Kanssanis, R., *Ann. Appl. Biol.*, 1957, **59**, 447.
33. Kartha, K. and Gamborg, O. L., in *Diseases of Tropical Food Crops* (eds Marite, H. and Meyer, J. A.), Universite Catholoque de Louvain, Belgium, 1978, p. 267.
34. Stevenson, G. C., *Genetics and Breeding of Sugarcane*, Longmans, London, 1965.
35. Krishnamurthi, M., *ISSCT Sugarcane Breeding News*, 1974, **35**, 24.
36. Krishnamurthi, M. and Tlaskal, J., in *Proc. Congr. Int. Soc. Sugarcane Technol.*, (*South Afr.*), 1974, **15**, 130.
37. Hu, C. Y. and Wang, P. J., in *Handbook of Plant Cell Culture*, vol. 1, *Techniques for Propagation and Breeding* (eds Evans, D. A., Sharp, W. R., Ammirato, P. V. and Yamada, Y.), Macmillan Publ. Co, New York, 1983, p. 177.
38. Barker, J. M., McInnes, J. L., Murphy, P. J. and Symons, R. H., *J. Virol. Methods*, 1985, **10**, 87.
39. Nelson, R. S. *et al.*, *Biotechnology*, 1988, **6**, 403.
40. Drake, L., *Biotechnol. News*, August 3, 1989, p. 8.
41. Rezaian, M. A., Skene, K. G. M. and Ellis, J. G., *Plant Mol. Biol.*, 1989, **11**, 463.
42. Mathews, B. F. and Schaeffer, G. W., in *Advances in Cell Culture* (ed. Maramorosch, K.), Academic Press, 1982, p. 267.
43. Broglie, K. E., Biddle, P., Cressman, R. and Broglie, R., *Plant Cell*, 1989, **1**, 599.
44. Guha, S. and Maheswari, S. C., *Nature*, 1964, **204**, 497.
45. Schwarz, D., *Mol. Gen. Genet.*, 1984, **196**, 81.
46. Tacchim, P. and Walbot, V., *Nestle Res. News*, Nestec Ltd. Vevey, Switzerland, 1987, p. 19.
47. Timm, M., *Biotechnology*, 1989, **7**, 118.
48. Ammirato, P. V., in *Handbook of Plant Cell Culture*, vol. 3, *Crop Science* (eds Ammirato, P. V., Evans, D. A., Sharp, W. R. and Yamada, Y.), Macmillan Publ. Co, New York, 1984, p. 327.
49. Ng, S. Y. C., *Plant Cell Tissue and Organ Culture*, 1988, **14**, 121.
50. Corley, R. H-V., Wong, C. Y., Wooi, K. C. and Jones, L. H., in *Conference on Oil Palm in Agriculture in the Eighties*, Kaula Lumpur, 1981.
51. Lutz, J. D., Wang, J. R., Rowe, J., Tricoli, D. M. and Lawrence, Jr. R. H., in *Tissue Culture in Forestry and Agriculture* (eds Henke, R. R., Hughes, K. W., Constantin, M. J. and Hollaender, A.), Plenum Press, New York, 1985, p. 105.
52. Rhodes, C. A., Lowe, K. S. and Ruby, K. L., *Biotechnology*, 1988, **6**, 56.
53. Redenbaugh, K., Nichol, J., Kossler, M. E. and Paasch, B., *In Vitro*, 1984, **20**, 256.
54. Ilker, R., *Food Technol.*, 1987, **41**, 70.
55. Shimomura, K., Satake, M. and Kamada, H., in *VI Int. Congr. Plant Tissue and Cell Culture* (eds Somers, D. A., Gengenbach, B. G., Biesboer, D. D., Hackett, W. P. and Green, C. E.), IAPTC, USA, 1986, p. 250.
56. Dougall, D. K., Labrake, S. and Whitter, G. H., *Biotech. Bioeng.*, 1980, **22**, 581.
57. Cords, J. M., Scorza, R. and Bell, R. L., in *VI Int. Congr. Plant Tissue and Cell Culture* (eds Somers, D. A., Gengenbach, B. G., Biesboer, D. D., Hackett, W. P. and Green, C. E.), IAPTC, USA, 1986, p. 146.
58. Strickland, R. G. and Sunderland, N., *Ann. Bot.*, 1972, **36**, 443.
59. Yamakawa, T., Ohtsuka, M., Onomichi, K., Kodama, T. and Minoda, Y., in *Proc. V Int. Congr. Plant Tissue and Cell Culture* (ed. Fujiwara, A.), Japanese Assoc. for Plant Tissue Culture, 1982, p. 273.
60. Yoshikazu, Y., Mixugochi, R. and Yamada, Y., *Proc. Int. Congr. Plant Tissue and Cell Culture* (ed. Fujiwara, A.), Japanese Assoc. for Plant Tissue Culture, 1982, p. 283.
61. Whitaker, J. R. and Evans, D. A., *Food Technol.*, 1987, **41**, 86.
62. Vaush, R., Ratilsky, M. B., Keren, Z. M. and Robenfield, B., in *VI Int. Congr. Plant Tissue and Cell Culture* (eds Somers, D. A., Gengenbach, B. G., Biesboer, D. D., Hackett, W. P. and Green, C. E.), IAPTC, USA, 1986, p. 116.

63. Carew, D. P. and Staba, E. J., *Lloydia*, 1965, **28**, 1.
64. Al-Abta, S., Galpin, I. J. and Collin, M. A., *Plant Sci. Lett.*, 1979, **16**, 129.
65. Turnbull, A., Galpin, I. J., Smith, J. L. and Collin, H. A., *New Phytol.*, 1981, **87**, 257.
66. Collins, H. A. and Watts, M., in *Handbook of Plant Cell Culture*, vol. 1, (eds Evans, D. A., Sharp, W. R., Ammirato, P. V. and Yamada, Y.), Macmillan Publ. Co, New York, 1983, p. 729.
67. Lindsey, K. and Yeoman, M. M., in *Primary and Secondary Metabolism of Plant Cell Culture* (ed. Neumann, K. H.), Springer-Verlag, Berlin, 1985, p. 304.
68. Townsley, P. M., *J. Inst. Can. Sci. Technol. Alim.*, 1974, **7**, 76.
69. Ravishankar, G. A., Rajasekaran, T. and Venkataraman, L. V., in *Proc. Natl. Symp. on Recent Advances in Plant Cell Tissue Culture of Economically Important Plants* (ed. Reddy, G. M.), Osmania University Press, Hyderabad, 1988, p. 119.
70. Diomande, M., *ATAS Bull. Tissue Culture*, 1984, p. 26.
71. Horowitz, R. M. and Gentilli, B., *US Patent*, 1983, **3**, 87.
72. Purhan, Z. and Martin, S. W., *Prog. Ind. Microbiol.*, 1971, **9**, 13.
73. Persions, G. J., *US Patent*, 1973, **3**, 723, 410.
74. Morris, J. A., *Manuf. Confect.*, 1972, **52**, 38.
75. Tahara, A., *Kegaku Kogyo*, 1972, **23**, 398.
76. Tahara, A., *Yakugaku Zasshi*, 1973, **93**, 951.
77. Handro, W., Hell, K. G. and Kerbau, G. E., *Planta Med.*, 1977, **32**, 115.
78. Furuya, T., in *Frontiers of Plant Tissue Culture* (ed. Thrope, T. A.), University of Calgary, Calgary, Canada, 1978, p. 191.
79. Arup, S., *Biotechnol. News*, 1989, **9**, 5.
80. Mathew, A. G., Lewis, Y. S., Jagadishan, R., Nambudiri, E. S. and Krishnamurthy, N., *Flavour Ind.*, 1971, **2**, 23.
81. Yeoman, C. M. M., Miedzybrodzk, M. S., Lindsey, K. and Mclaunchlna, W. R., in *Plant Cell Culture: Results and Perspectives* (eds Sala, F., Parisi, B., Cella, R. and Giferri), Elsevier, New York, 1980, p. 327.
82. Ravishankar, G. A., Venkataraman, L. V. and Kadyan, A., *Curr. Sci.*, 1988, **57**, 679.
83. Ravishankar, G. A., Rajasekaran, T., Sarma, K. S. and Venkataraman, L. V., in *Proc. Symp. Plant Cell and Tissue Culture of Economically Important Plants* (ed. Reddy, G. M.), Osmania University, Hyderabad, 1987, p. 110.
84. Sujata, V., Ravishankar, G. A. and Venkataraman, L. V., *Appl. Biochem. Biotechnol.*, 1990, **12**, 336.
85. Watanabe, K., Yano, S. and Yamada, Y., *Phytochemistry*, 1982, **21**, 513.
86. Pais, M. S. *et al.*, in *VI Int. Congr. Plant Tissue and Cell Culture* (eds Somers, D. A., Gengenbach, B. G., Biesboer, D. D., Hackett, W. P. and Green, C. E.), IPATC, USA, 1986, p. 69.
87. Ravishankar, G. A., Rajasekaran, T. and Venkataraman, L. V., *Pyrethrum Post*, 1989, **17**, 66.
88. Shannon, J. C. and Garwood, D. L., in *Starch: Chemistry and Industry* (eds Whistler, R. L., Paschall, E. F. and BeMiller, J. N.), Academic Press, New York, 1984, p. 26.
89. Lawrence, R. H., *Cereal Foods*, 1987, **32**, 759.
90. Flavell, R. B., Payne, P. I., Thompson, R. D. and Law, C. N., *Biotech. Bioengg. News*, 1984, **2**, 157.
91. Kunimoto, L., *Biotechnology*, 1986, **40**, 58.
92. Sharp, W. R., *J. Am. Oil Chem. Soc.*, 1986, **63**, 594.
93. Fujita, Y., Hara, Y., Suga, C. and Morimoto, T., *Plant Cell Rep.*, 1981, **1**, 61.
94. Tisserat, P., *Biotechnology*, 1987, **5**, 10.
95. Himeno, H. and Sano, K., *Agric. Biol. Chem.*, 1987, **51**, 2395.

RESEARCH ARTICLE

A computer simulation model for action potential in an excitable membrane

Minal P. Mujumdar and Chanchal K. Mitra
School of Life Sciences, University of Hyderabad, Hyderabad 500 134, India

We present a computer simulation of action potential, developed in most cell membranes but a characteristic property of the neuron membrane. The simulation program is a model developed theoretically to see the generation of action potential once the nerve is stimulated, using values from the literature and calculated values. The effects of different factors, such as rate of diffusion, slopes of the permeability curves used to determine the permeability of the different ions, and strength of the external stimulus applied, on the action potential are also seen. The best-suited values for better results are then chosen. The basic interest is to study, theoretically, the various aspects of action potential generation and the restoration of resting potential, and to compare the results with those of experimental studies.

IN a living cell a potential difference exists between the cytoplasm and the extracellular environment, with the inside of the cell generally being negative with respect to the outside. This potential is called resting potential and is caused by the unequal distribution of ions in the inside and outside solutions on either sides of the plasma membrane surrounding the cell and by differences in ionic permeabilities. The neuron membrane is capable of generating a nerve impulse (transient potential change) and has a unique structure, the synapse, for transferring information from one neuron to another, when stimulated.

When the nerve impulse starts, after having been triggered, the voltage across the membrane is lowered locally. Immediately ahead of the electrically altered region (in the direction in which the nerve impulse is propagated) channels in the membrane open and let Na^+ ions pour into the axon.

The process is self-reinforcing: the flow of Na^+ ions