PLANT BREEDING

Parasexual Tobacco

by our Botany Correspondent

A FURTHER step towards a means of producing hybrid plants without recourse to sexual reproduction has been reported from Brookhaven National Laboratory in New York. Three members of the department of biology have grown viable plants from fused cells of two species of tobacco, *Nicotiana glauca* and *N. langsdorffii* (P. S. Carlson, H. H. Smith and R. D. Dearing, *Proc. US Nat. Acad. Sci.*, **69**, 2292; 1972). Thus they have combined the previous Japanese and British achievements, when isolated protoplasts—the naked contents of plant cells—were induced, on the one hand, to grow into whole plants, and, on the other hand, to fuse with protoplasts from another plant without further development.

The Brookhaven group used similar techniques to obtain and fuse protoplasts from the two species of *Nicotiana*. Strips of epidermis from young leaves of each species were incubated in an enzyme mixture containing cellulase, macreozyme and sucrose to dissolve away the cell walls. Protoplasts were collected by low speed centrifugation, and then the two species were suspended in sodium nitrate solution, which had previously been shown to induce fusion.

After thirty minutes a peelet was spun down, resuspended and plated on a nutritive medium. At this stage Dr Carlson and his colleagues had a mixture of fused hybrid protoplasts and unfused parental protoplasts of both species. The methods used to select out the hybrid cells were based on knowledge of the biochemical characteristics of the hybrid *N. glauca* × *N. langsdorffii* which had already been produced by orthodox sexual means. Regeneration media were used on which only the cells containing the genetic information of both parents would be able to grow.

Out of more than 10^7 protoplasts of each parental species, thirty-three hybrid cells developed into calli which produced rudimentary shoots and leaves but no roots. Further development was obtained by grafting the regenerated shoots onto cut stems of young *N. glauca* plants.

Morphological and biochemical characteristics of the resulting plants, and the flowers and seeds they have now produced, compared exactly with those of the sexual hybrid between the two tobaccos. This confirmed that the parasexual methods used had yielded true hybrids. Of course the next goal in this work is to develop hybrid plants from parents that cannot be mated in the conventional manner, but nevertheless have characters that plant breeders would like to see combined. At present, however, there are technical problems to be overcome. The exact conditions have to be found for the regeneration of the fused protoplasts, and there is also the problem of distinguishing hybrid from parental tissue after growth has been induced. It will be interesting to see the next development in this branch of the new botany.

HISTONES

In vitro and *in vivo*

from our Cell Biology Correspondent

DURING the past several years a class of RNA molecules which sediment at 7-9S has, with increasing confidence, been claimed to contain messenger RNAs for histone proteins. These putative histone mRNAs have several properties that distinguish them from other putative and identified messengers. For example, they appear to lack the 3′ terminal tracts of polyadenylic acid present in many other messengers; they are transported from the nucleus to the cytoplasm more rapidly than most other RNAs; they appear in small cytoplasmic polysomes only when the cell is replicating its DNA, and they seem to be specified, at least in sea urchin cells, by reiterated and clustered DNA sequences. But, of course, none of these properties of putative histone mRNAs prove that these molecules actually specify histones, which explains why the report of Jacobs-Lorena, Baglioni and Borum (*Proc. US Nat. Acad. Sci.*, **69**, 2095; 1972) will be greeted with signs of relief in many quarters. For they have shown quite conclusively that the 7-9S putative histone mRNAs isolated from the polysomes of populations of synchronized HeLa cells in S phase are indeed translated in a cell-free system obtained from mouse ascites tumour cells to yield polypeptides which include all five classes of human histones.

The 7-9S polysomal RNAs extracted from HeLa cells stimulated protein synthesis in the ascites cell-free system from four- to eleven-fold; the histones were identified amongst the products of translation by electrophoresis in SDS and acidic polyacrylamide gels, and by comparing tryptic peptides obtained from the polypeptides made *in vitro* with those obtained from authentic human histones. Whether or not the 7-9S RNA, as prepared by Jacobs-Lorena *et al.*, contains messengers other than those for histones, and whether or not RNAs other than messengers are present, remains to be seen. The fact that the 7-9S RNA from HeLa cells failed to stimulate appreciably incorporation of tryptophan, an amino-acid absent from histones, suggests that the histone mRNA was not extensively contaminated with mRNAs for other proteins, but the possibility that it contained messengers for a non-histone protein deficient in tryptophan cannot be excluded. The fact that the histone mRNA preparations had less messenger activity in the cell-free system than preparations of haemoglobin mRNA may mean that RNAs lacking messenger activity were present as contaminants, but such differences in messenger activity, which is assayed *in vitro*, can be explained in several other ways; for example, histone messenger may be more labile than haemoglobin messenger.

Once a histone polypeptide chain has been synthesized, several of its lysine residues may be acetylated, and, as Candido and Dixon report (*ibid.*, 2015), although the sequence of the first

Selecting Haploid Plant Cells

BACTERIA lend themselves to sophisticated genetic analysis for several reasons, not the least of which are that they are haploid and they multiply quickly. Genetic analysis of higher plants and animals, by contrast, is hindered by the much longer life cycle and by the diploid nature of the somatic cells. If somatic cell geneticists could at will produce and maintain haploid cell lines, half their battle would be over, which explains why the report of Gupta and Carlson in next week's *Nature New Biology* (September 20) should be welcome reading for many.

Parafluorophenylalanine has for the past several years been used occasionally to induce the haploidization of one or two species of different genera of fungi, but Gupta and Carlson appear to be the first people to exploit this compound to select and maintain cultures of haploid cells from higher plants. As a model system they established in culture cells of haploid and diploid strains of tobacco plants, and then added various concentrations of parafluorophenylalanine to the culture media. At concentrations which kill a callus of diploid cells a haploid cell callus continues to grow vigorously. It should therefore be possible to use this amino-acid to select haploid cells from populations of cells of varying ploidy, such as emerge in anther cultures. To date, of course, Gupta and Carlson have used only tobacco plant cells, but if the effect of parafluorophenylalanine is not specific to this species they may well have hit upon a generally useful method of obtaining haploid, higher plant, cell cultures.