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A lovely clone of coconuts

Coconut palms can now reproduce in the test-tube, thanks to advances in tissue culture. At last we can produce reliable varieties of the "tree of life"

Richard Branton and Jennet Blake

PALMS are fascinatingly diverse. Some of the largest and most distinctive leaves, flowers and seeds in the plant kingdom are borne by the 2600 species that are spread throughout the world. They are also one of the most useful groups of plants known to humankind, second perhaps only to the cereals and grasses. For centuries palms have provided the people of tropical and sub-tropical areas with food, drink, fuel and shelter; the coconut palm in particular is with some justice known as the "tree of life". Palms are now also commercially important, but production is undermined by the variable quality of cultivated palms. A solution is at hand: for botanists have just found a way to produce clones, millions of replicates of a single palm, by persuading ordinary cells to develop into plants in tissue culture. This year the first clone of a coconut palm was born in a British laboratory.

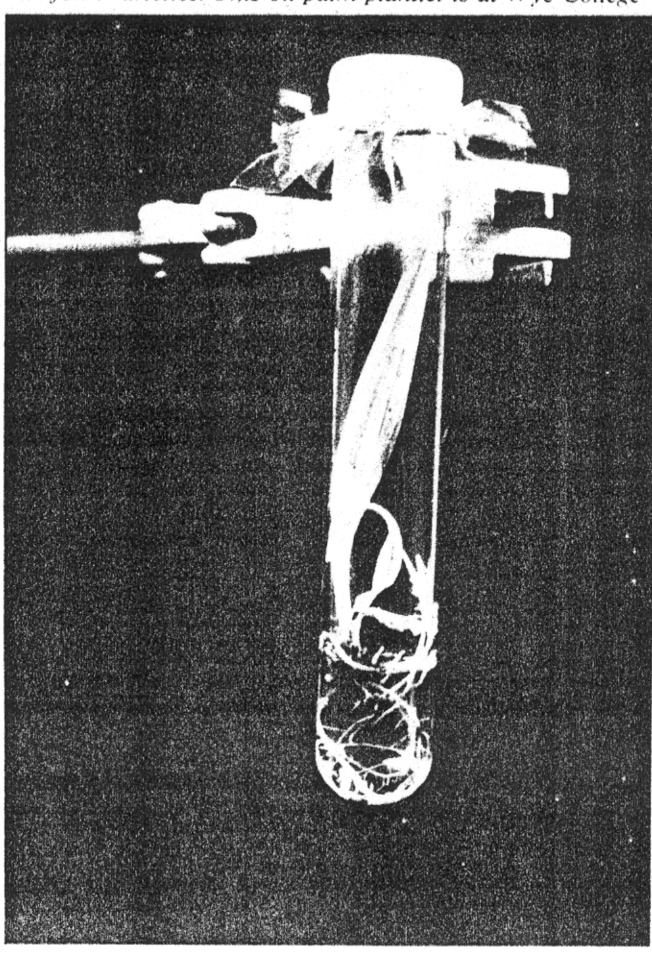
Of all the useful palms, three species are valuable commercially. The date palm (*Phoenix dactylifera*) is widely cultivated in the arid regions of North Africa and the Middle East where its fruits provide a carbohydrate food. Most important on a world-wide scale, however, are the oil palm (*Elaeis guineensis*) and the coconut palm (*Cocos nucifera*). The fruits from both provide valuable oil that ends up in margarine, cooking oil, soap, detergents, cosmetics, pharmaceutical products or fuel. The coconut palm also provides copra, the white flesh inside the seed, which is turned into desiccated coconut mainly in Sri Lanka, and widely used in the confectionery and bakery trades. Even the husks are useful: for coconut matting or as a significant source of fuel.

The demand for palm products has risen so much since the beginning of the 19th century that many producing countries are now unable to meet even their home requirements; only a few, notably Malaysia and the Philippines, are able to produce for export. Part of the problem lies in the difficulty in producing large numbers of palms that will give high yields and be resistant to diseases. Coconut and oil palm can be propagated naturally only from seed; a grower cannot take cuttings, since each palm has only one vegetative growing point. The trouble with growing from seeds is that palms do not breed "true to type"; the offspring differ greatly in vigour, productiveness and resistance to disease. But these traits are often not expressed until the palms are 10 years old, and the commercial life of a coconut palm may be as long as 60-70 years. So selecting parent palms and breeding new hybrids can take decades. The variable quality of palms grown from seeds may not be a serious problem for large plantations but for the millions of smallholders throughout the tropics, any reduction in yield, through disease, for instance, seriously reduces their standard of living.

Since the early 1960s, governments and commercial firms have been investigating tissue culture as a way of producing scores of genetically identical palms. The idea is to persuade cells from a leaf, for instance, to develop into complete individual plants. That way, each plantlet could contain the same genes as the mature donor palm; the whole gamut of genetically identical offspring collectively constitute a clone.

It has not been easy to clone palms. Unilever (UK) and the French IRHO (Institut de Recherches pour les Huiles et Oléagineux) have spent a great deal of time and money on research and development of oil palms, but it has taken them nearly 10 years to produce the first clonal plantlets from oil palm. After some 13 years' work, we at Wye College (University of London) have now produced a clonal plantlet from the coco-nut. Competition and secrecy have also been great, since a successful technique applied on a large scale could realise huge profits. Unilever has recently built a "tissue culture factory" in England with a potential capacity for producing half a million clonal oil palms a year for export, and also has an identical unit in Malaysia, in association with Harrisons and Crosfield.

Like the coconut, the oil palms too must be cultured to produce uniform varieties. This oil palm plantlet is at Wye College



A. Brackpool

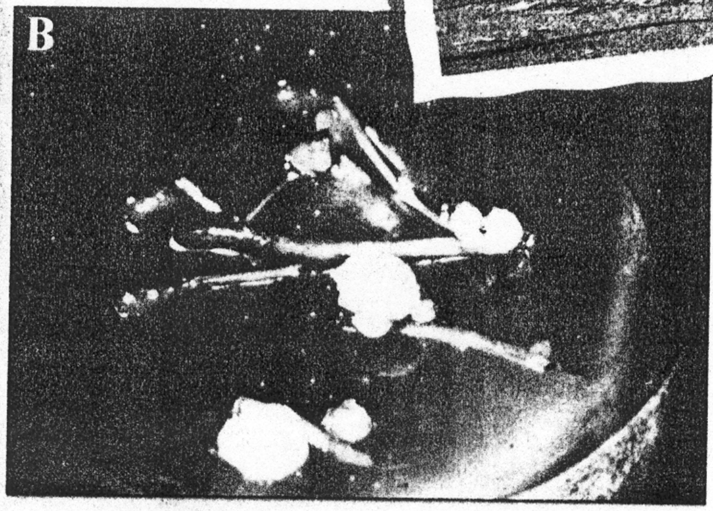
To clone palms we start by removing a small piece of living tissue, the explant, from the donor palm, ideally without destroying the tree. Living cells from coconut palms are brought by air from Jamaica. We have used various parts of the mature palm for explants, such as secondary or tertiary roots, immature flowers and spear leaves. Even the smallest explant contains thousands of living cells, each containing all the genetic information needed to produce an exact copy, or clone, of the donor palm. Before culturing, we sterilise the surface of the material with a dilute solution of bleach; roots, which are heavily contaminated with microorganisms, need to be treated initially with mercuric chloride. The treated explants are then placed onto the surface of a sterilised medium which is solidified with agar and contains essential nutrients—sucrose, mineral elements, vitamins and amino acids.

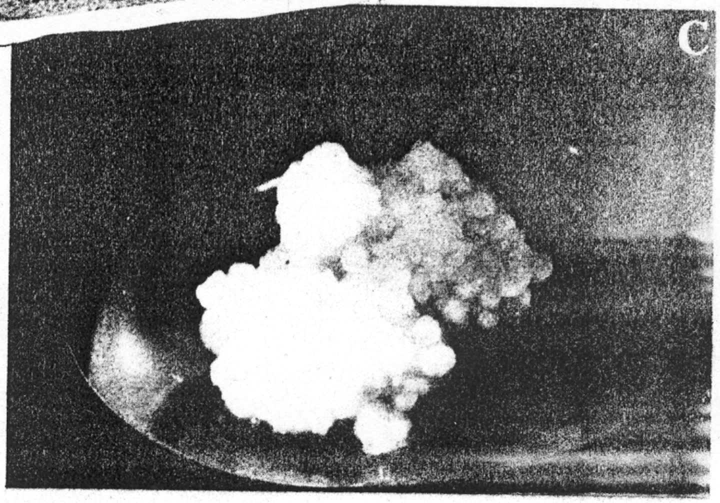
Plant growth regulators—hormones such as auxins and cytokinins—are also added to stimulate the cells to divide and produce a mass of "undifferentiated" cells. Such a mass is called a callus; a plant

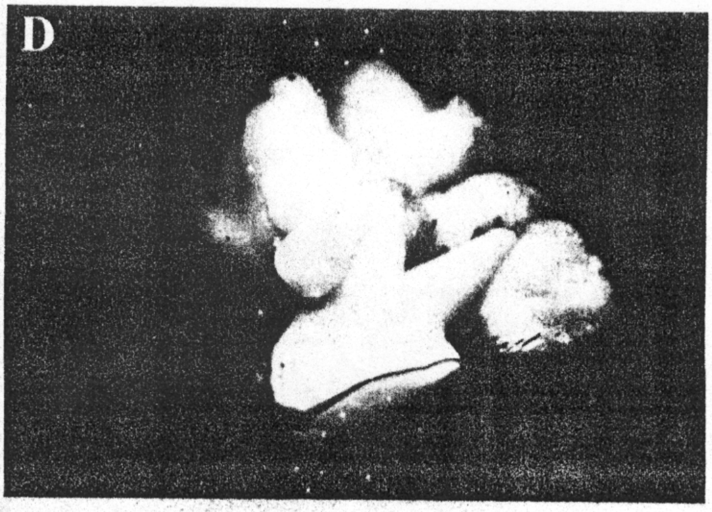
*Culture of a coconut. Roots taken from a mature coconut palm (**A**) are treated with hormones in the laboratory to encourage a clump of undifferentiated cells, the callus, to form (**B**). The callus is next removed from the roots and grown (**C**). After some months a large embryoid—an embryo plant—is formed (**D**), which develops into a clonal plantlet—with shoots, roots and, at the base of the shoot, the haustorium (**E**)*

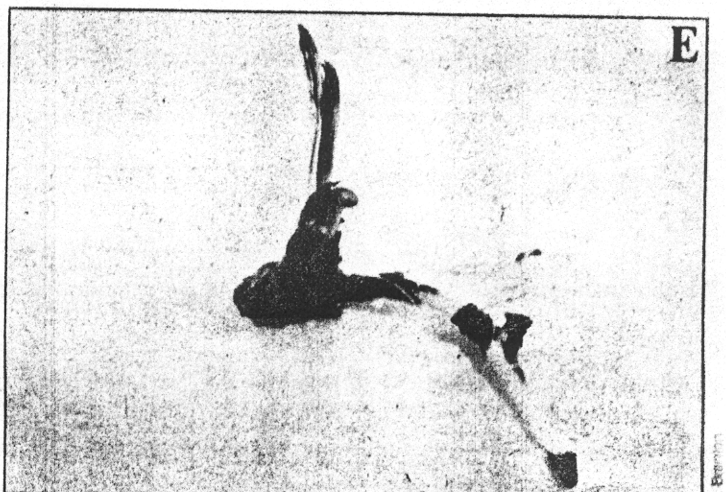
naturally produces callus when it is wounded. Optimising the concentrations of all the ingredients in the medium has taken years of research. We find, for instance, that high concentrations of the hormone 2,4-D encourage the callus to form, but activated charcoal must also be added to the medium to prevent the chemical killing the cells. Only in the past three years have we been able to grow a callus to the point where the cells can be successfully multiplied by sub-culture. At this stage the callus is a compact mass of small similar cells (with dense cytoplasm) showing little sign of differentiation.

The cells have the potential to return to the embryonic state and develop into complete plants but the high level of auxin needed to encourage the callus to grow actually inhibits the next stage—the development of embryogenic cells. For the past three years we have been altering the concentrations of growth regulator to try to stimulate the callus to form so-called somatic embryos or embryoids. These embryoids are analogous to the normal embryos in the seed but contain only genetic material from the single parent—that is, they are clonal. In the early days of our research, many abnormal structures were









Each coconut in this splendid crop could develop onto a new tree—but each new tree would be different from all the others. The present "varities" do not breed true

formed—it was easy to produce perfect roots but no shoot!

Problems arose when we reduced the concentration of auxin to try to stimulate the development of embryoids. The normal seed embryo of the coconut germinates to produce not only a shoot and a root but also a very large feeding organ or haustorium (cotyledon) which eventually fills the cavity of the nut; it absorbs the copra and feeds the developing plant. The coconut embryoids we produced in culture also have a haustorium, but if the auxin is withdrawn too early, this haustorium continues to expand and the shoot fails to develop.

We have overcome this problem by gradually reducing the type and concentration of auxin so that shoot-like structures with roots develop. The first clonal coconut plantlet was produced in our laboratory from flower tissue received in July 1982, after some 8 months in culture. Coconuts appear to regenerate more quickly than oil palms; it takes between 9 and 24 months to regenerate oil palm plantlets from initial culture. Incidentally, could our plantlet be the result or cause of a Sheldrake effect (*New Scientist,* vol 90, p 766)? Many thousands of plantlets can now be produced (at least in theory), each identical to the donor palm in yield or resistance to disease, thus providing growers with uniform, high quality material.

But the technique is still fraught with problems. Different genotypes respond differently in culture, and the initiation of callus on the initial explants is slow and erratic. In the early stages, the callus grows slowly and the formation of embryoids is unreliable. Once embryoids are induced, a proportion may be abnormal. Finally, the whole process takes months, if not years.

But technical problems aside, the most important question is "Will the plantlets be identical to the donor palm?" At each cell division there is always a chance of genetic change, and some of the hormones used, such as 2,4-D, may induce abnormalities. Unilever has begun extensive field trials of clonal plantlets of the oil palm; its initial results suggest there is no significant variation, but it will still be some years before genetic stability within clones can be assessed.

Cloned palms may increase yields from oil palms by 20 to 30 per cent and yields from coconut could be as high as five-fold over unselected stock; 1·2 tonnes/hectare of copra from traditional "varieties" could be increased to 6 t/ha. In Kerala, India, an important producer of coconuts, the average yield is 35 nuts per palm per year. Yet single, "elite" palms have been identified which produce yields of over 400 nuts per year. It will soon be possible to clone such superior palms. Low soil fertility may still make high yields elusive, but even a small increase in yield could be important on holdings of less than 5 hectares. Improved yields would not only increase the production of oil and copra but also the harvests of husk and shell which are immense potential sources of energy; the 1978 Philippine harvest of over 12 million nuts is equivalent in terms of energy to 3·8 billion litres of petrol (31×10^{12} kcal).

The future of palm tissue culture does not stop at merely producing millions of clonal plants. Continuing progress in the field of genetic engineering should soon enable scientists to carry out "breeding" within the test-tube. New genetic information could be introduced directly into the cell using vectors (see p 550), or cells of different chosen strains could be fused. Such processes may dramatically reduce the time required to produce new varieties; present breeding techniques may take 50 years to produce a desired hybrid. It is not easy to store seeds as large as a coconut, but soon it should be feasible to preserve just the germplasm as embryoids in a deep-freeze and later grow a complete plant in tissue culture. Millions of "potential palms" could be stored in a few test-tubes.

Viruses and mycoplasmas responsible for serious diseases in the coconut may also be eliminated using the techniques of tissue culture. The low average yield in Kerala is partly due to diseases such as to root wilt. Some of the high-yielding palms already identified are also resistant to this disease, and are thus ideal candidates for clonal propagation. Another disease, lethal yellows, can rapidly wipe out whole plantations in the Caribbean. Disease-resistant hybrids produced by the plant breeder cannot be multiplied satisfactorily by seed because of the variable nature of this palm, but clonal propagation could be a powerful tool for building up breeding lines, and for producing hybrid stock for planting in the field.

The production of clonal oil and coconut palms have been major achievements. These techniques may not be properly exploited much before the end of this century, but the future of all concerned with palms, from the large plantation owner to the thousands of smallholders, is promising. Commercial interests will ensure that clonal plants of the oil palm are successfully exploited, but it will need government support to ensure that benefits from clonal coconuts reach the millions of smallholders whose lives depend on the success of their palms. And while technology will be able to provide the best palms it will inevitably be left to the growers to realise their full potential by good husbandry. □

Dr Richard Branton and **Dr Jennet Blake** are Research Fellows at Wye College (University of London). They wish to thank Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) for its support, the Coconut Industry Board, Jamaica, for palm material, and John Eeuwens for much of the early research on coconuts.