PLANT BIOTECHNOLOGY

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Soon after completing installation for basic plant tissue culture we initiated the first of our programmes on the zygotic embryo culture of certain meliaceae for reforestation.

Meliaceae embryo culture

Seeds from mature and immature fruits of Azadirachta indica (kohomba) and Melia composita (lunumidella) collected from various locations were dissected to determine the most suitable manipulation for excising embryos. In fact, seed anatomy is quite specific for each species and the procedure has to be formulated afresh whenever a new species is taken up for study. The optimal culture medium has also to be determined for each species. Since media are usually made up of about thirty constituents, each one at a critical concentration, an empirical determination would entail trying out an impossibly large number of tentative media at first. Thus, in the scheme of broad-spectrum trials as proposed by de Fossard, about 4000 different preparation have to be assayed before further improvement to obtain the ideal medium. However, by grouping constituents into categories and varying the concentration of categories to a minimum, preliminary trials can be done in manageable series. Thus, with three categories (e.g. inorganic salts, carbon compounds and phytohormones) and two concentrations (high and low) only 8 trials are necessry (i.e. 2). If constituents are grouped into five categories (inorganic macro elements, micro elements, carbon sources, vitamin like factors and phytohormones) 32 media have to be prepared (i.e. 2) and if their concentrations are to be assayed at three levels (high, low and zero) then 243 are media required (i.e. 3). Using this strategy we divised media which induced 90% germination of the excised embryos of lunumidella and 100% of kohomba (Details of excision procedures and germination *in vitro* are given in Ms. Gunasekera's report).

Germplasm conservation by inducing slow growth

Paradoxically, once embryos grow profusely on optimal media, it is often desirable to slow down their growth artificially for practical purposes, such as when planting stock has to be held over for several seasons later, or when planting operations in a forest are delayed several months for reasons beyond our control. Embryos should be maintained *in vitro* as healthy as possible, with only their actual growth rate reduced to a minimum, which may be achieved by reducing the temperature or by devising specific slow growth media. We have adopted both low temperatures and the incorporation of mannitol in place of sugar in the culture medium. Mannitol is a carbohydrate which cannot be assimilated by plant tissues; it however maintains the osmolality and other physico-chemical properties of the medium so that tissues are not harmed while being deprived of their energy source. This and the reduced temperature bring down metabolism to a basal rate until such time as the temperature is raised and sucrose is provided to restore normal rate of growth (Details of slow growth in the presence of mannitol and at low temperatures appear in Ms. Gunasekera's report).

Albinism

A significant number of lunumidella embryos developed a form of albinism *in vitro*, that is, the first two leaves were white and devoid of chlorophyll. This is a somewhat widespread and ill-understood phenomenon particular to certain species. It occurs in androgenetic embryos (haploid progeny from unfertilized pollen) of some cereals like wheat and rice and has also been reported in the chestnut, but never in other families like the Solanaceae. A tentative molecular explanation is that the chloroplasts of albino leaves lack ribosomal RNA following an extra-nuclear mutational event during androgenesis, but this cannot yet be proved for want of sufficient albino material to test with the appropriate DNA probes. Chinese workers claim that the cause is purely phenotypical and can be remedied by suitable modifications of nutrients. (Ms. Gunasekera's report will show that albinism in lunumidella may be correlated with the stage of maturity of the excised embryos. Hopefully, a sufficient number of albinos may be prepared in the future for looking into their RNA and DNA).

Jacaranda and Nutmeg

We have added the embryo culture of the Jacaranda and the Nutmeg to this programme. The Jacaranda is one of the six ornamental trees with which we hope to border the Institute grounds. Its propagation by embryo culture has presented no particular difficulty, and we shall apply the same technique the next season to another of the six species, Tabebuia, which is also a Bignoniaceae.

The natural germination in the soil of nutmeg seeds is extremely poor. From our observations it appears that the hard ruminated endosperm in which the embryo lies is very susceptible to fungal infection during maturation. Very young seeds are however free from infection and are furthermore soft and convenient to dissect. But at this stage embryos are so juvenile that they require a medium heavily supplemented with nutrients. We were able to obtain growing plantlets of nutmeg under sterile culture; these are intended for Brazilian scientists who will provide us, in exchange, germplasm of one of their species *Anacardium microcarpa* which is a particularly a drought-resistant relative of the cashew and not found in Sri Lanka (Details of Jacaranda and Nutmeg appear in the report of M. Iqbal and S. Ramanayake).

Bamboo

The second of our programmes involves the propagation of the giant bamboo, *Dendrocalamus giganteus* by *in vitro* methods. The choice of explants, difficulties of surface sterilization and the growth response on various culture media are given in the report of M. Gunasekera. One of our immediate aims is to obtain the multiplication of shoots from axillary buds by using high cytokinin in the medium as reported elsewhere by Chaker-Barzanov for a temperate bamboo; Pseudosasa japonica. He was however unable to induce roots in them and we are also confronted with the same difficulty in Dendrocalamus. Dr. Chaker is expected to join our group in the IFS later in the year.

Introduction of Forest tree germplasm

One of our long-term projects concerns the 900 odd tropical forest trees from all over the world whose edible fruit are under-utilized at present. About 80 of these are found in Sri Lanka, while there is no reason to think that the others will not thrive here if introduced, since all types of tropical habitats are found in some part of the island or other.

Our technique of explanting in the field and transporting sterile cultures has been described previously. It provides one of the best phyto-sanitary guarantees known so far. We made use of the opportunity of a recent visit to Kenya to collect about twenty species of forest fruit trees. All of them were species which we saw for the first time. Very often we had much difficulty in deciding on suitable sampling and dissection procedures on the spot in the forest itself. After transport of *in vitro* embryos to Sri Lanka, some species are now growing in pots or poly bags and will soon be transplanted to a suitable location here. This our first and though a badly prepared attempt to introduce germplasm from a distant country, is quite encouraging for future expeditions.

Winged bean

This is our latest programme. A general objective is to produce a bush-type of plant instead of a straggling creeper. Obviously such a spontaneous mutant is being widely searched for, and attempts have also been made to induce mutations by irradiation and chemical agents in other laboratories.

One of our approaches is to obtain androgenetic plants, which will necessarily be homozygous. All recessives will therefore be expressed and there are chances that a bush-type recessive which had remained masked right throughout the evolution of the winged bean from a wild ancestor may come out for the first time. When we cultured anthers to obtain embryos from the pollen it was however the surrounding tissue that grew into a callus (see report of M. Iqbal and S. Ramanayake). Therefore the very small microspores will have to be isolated from the surrounding tissues before culturing in the next attempts. Incidently, the callus we obtained can be maintained for use as a substrate for mutation studies now.

To isolate microspores of the right stage, which is generally the uninucleate stage just after the first mitosis following meiosis, we examined cytological preparations and correlated this stage with the size of the bud. Buds of length 3.5 to 4.0 mm seem to be the ones in which microspores are in the proper stage. Our next trials will therefore involve the micro-dissection of the minute anthers of these buds at the right time [Cytological observations are described in the report of M. Iqbal and S. Ramanayake].

Apart from the genotypic selection of a bush-type, manipulation of the phenotype by in vitro micrografting could also lead to the same result. But this will require more skilled effort. The best scion for micrografting is indeed the dome of the apical meristem which rarely exceeds 0.1 mm, but which carries the morphogenetic potential of the whole shoot. Unfortunately, like in all the Papillionaccae, the apex of the winged bean is covered tightly by several successive large stipules of the youngest leaves. Removing them with a microscalpel damages the fragile dome invariably. We have nevertheless found that when the epicotyl of a germinating seedling is decapitated, the dormant buds in the axils of the two cotyledons (cotyledons are morphogenetically equivalent to leaves) could develop sometimes in a matter of hours. These buds are indeed naked shoot meristem and are easily excised and will be used as scions for grafting (see also report of M. Iqbal and S. Ramanayake).