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PRELIMINARY REPORT ON A TECHNIQUE WHICH PROVIDES A "MATURITY FACTOR" FOR TREES GROWN IN TISSUE CULTURE

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I would like to preface this paper with a quote from Dr. Ron de Fossard. He advises that:

"We should not lose sight of the advantage of tissue culture plants and propagation. It is not just to clonally propagate a cultivar. It is to produce a *far superior* product, free of virus, fungi, and bacteria, from a highly desirable horticultural specimen and, where yield is important, from the upper 0.1% or better of the normal curve of distribution of the species."

Good and timely advice, indeed. Anyone can produce a plant in tissue culture. Often, a little careful juggling with media can produce better yield results than those published in the literature — but, to what end? Many of the plants grown in culture originate from seed or spores. Frequently, too, tissue-grown plants are just that, and *no positive selection* has actively taken place. Consequently, these plants are of little or no value in improving the standards of that cultivar. I feel it is an essential feature of any commercial tissue culture lab to actively improve the quality of those plants chosen for culture.

Dr. de Fossard goes on to say:

"It (the tissue culture plant) should be able to outsell plants produced by other methods of propagation because it should yield a *more valuable plant* and thus sell for a higher price. It should permit all-year round propagation. It should permit the propagation of species that cannot be vegetatively propagated by any other means. It should lead to the exploitation of protoplast and haploid work. It should enable clean plants to be kept clean more easily than at present. It should enable the expedition of plants from one country to another. It should give us high multiplication rates."

All excellent points. However, there is one more feature that a cultured plant *could* give us. That is, *a precocious and predictable yield of fruit or flowers.*

I believe that we have developed a working hypothesis which offers a very real advance in the practical utilization of tissue culture techniques. This advance utilizes that final point — "a precocious, predictable yield of flowers or fruit."

You will see from the title of this paper that it is a discussion of a potential technique. I must stress at this time that it is a laboratory technique only; one that is supported only by experimental evidence, and *not* by any large scale field trials.

I describe some of the results here and detail some aspects of my work. It is hoped that field proof and amplification of the technique will be available for the 1983 Darwin I.P.P.S. Australian Conference.

We are told by most of the conventional tissue culture exponents that an ability to rapidly multiply is a feature — an essential feature, of juvenility — conversely we are told that mature (i.e. sufficiently old fruit-bearing plants), particularly in the woody angiosperms, cannot or will not multiply "in vitro".

This is not strictly true! Under the right conditions, mature tissue can be induced to multiply "in vitro" and, in fact, can be expected to behave in a juvenile manner. As a corollary, in our experience, this tissue, when removed from culture, can *revert back* to the *mature status* of the donor parent.

It is unfortunate that most of the rapid multipication techniques used in horticulture concern soft-tissued plants. It shows a regrettable lack of insight that much of the early work done on woody plants merely adapted those techniques used for soft tissue material. Almost invariably this meant using juvenile (i.e., seedling) or quasi-juvenile (i.e., regenerated juvenile shoots from wounds on mature plants), as starting material to get any sort of response.

Techniques such as callus formation and subsequent redifferentiation of leafy shoots seemed to be most popular, closely followed by callus/embryoid production. Both techniques meant that any regenerated plantlets would be completely juvenile in morphology, behavior, and general characteristics. This, in turn, meant that they would follow a "normal" development to maturity over a period of time.

Where de-differentiation into callus, and subsequent redifferentiation into leafy plants occurs, all mature characteristics of the tissue are apparently lost.

There are a significant number of references in the literature which indicate that "sometimes" and under "certain" conditions, regenerated plantlets did not completely revert to a juvenile state, but retained some, most, or all observable mature characteristics.

Our technique depends on the development and multiplication of axillary shoots, and complete avoidance of any callus phase.

Within the scope of our work, I have defined "maturity" on a purely morphological basis, being:

(i) The immediate production of adult leaves, (if they differ from juvenile leaves) on being deflasked.

(ii) The ability to produce 'de novo' flower buds, or bear fruit in a shorter period of time than the equivalent "normally" propagated plant.

(iii) A reduced rate of growth, with more emphasis on flower buds or fruiting wood than conventionally propagated plants of the same age.

If I may digress a little, at this time, imagine the situation that could exist in the future. To an orchardist or farmer, the knowledge that stock planted out was semi- or wholly- *mature, at the time of planting* would be a unique advantage.

Consider:

(i) that such stock could commence bearing at an early age, obviating a long lead-in time for yield, and incidentally, for profit.

(ii) that such stock, because of its precocious nature or semi-mature status would not necessarily be as vigorous as seedling, cutting grown, or grafted plants. This could give benefits such as a tree of reduced size and could reduce the need for regular pruning, and

(iii) such a condition would generate great advantages when harvesting a crop — harvest could be achieved quicker and more cheaply if pickers worked from the ground or mobile picking units, rather than having to climb trees.

Our lab, over the last 2½ years has worked on a number of woody plant species. Our most spectacular results have been with roses and grapes. Other trials have been conducted with cassava, papaya, mango, bougainvillea, grevillea, and passion fruit. In the United Kingdom and Europe, I had the opportunity to test some of my ideas on such diverse plants as coconut, durian, and apple.

Other workers, working independently have published results that seem to confirm the existence of the condition I have called the "*maturity factor*".

Such indications occur with coffee, apples, peaches, and cherries. In fact, it appears that any plant which can be successfully marcotted (air-layered), can be successfully shown to exhibit the "maturity factor".

Expressed simply, this "maturity factor" states that: "providing the donor plant is mature and in a suitable condition before excision of axillary buds, those mature buds may be induced to behave in a juvenile manner "in vitro", yet return to a mature or semi-mature condition after deflàsking, in a relatively short time."

We have had miniature and floribunda roses flower from culture in 49 days after deflasking. Hybrid teas take a little longer, generally around 60 to 65 days to full opening of the blooms.

With the smaller-flowered types the blooms are generally identical with those produced on cutting-grown or grafted plants. The hybrid teas generally have only half the number of petals of a 2-year-old field-grown plant. However, in the second flush of flowering blooms are virtually normal and plant growth is similar to that of a grafted plant.

Roses are not a field crop in the tropics, they're not even a good garden subject, as our constantly warm weather provides no chilling to terminate a period. To test the plant completely then, we are setting up field trials in Adelaide, Melbourne, and Townsville. Those results and independent assessments will be available at the Darwin IPPS Conference in 1983.

Grapes are in a similar situation. We have successfully cultured axillary buds, tendrils, and stem segments and achieved commercial propagation rates from all sources. Plants raised *in Darwin have flowered and set fruit* in as little as 12 weeks from deflasking. Once again though, independent testing in a more suitable climate needs to be done for a complete assessment.

Similarly, we have achieved flowering (but aborted fruit, soon after set) on papaya at an age of only 12 weeks from the flask. Since the initial trials on random stock, we have been plagued with bacterial problems. These are mostly a diptheroid as well as *Pseudomonas putrifaciens*, which seem to be intimately associated with the clone lines we are testing on behalf of the local Department of Primary Production. We seem to have gained control of the infections now, using chloramphenicol succinate (an antibiotic of sinister reputation — extended exposure in humans destroys bone marrow). However, at 1 to 5 ppm it knocks out the pathogens and scarcely damages tissue.

Israel has reported precocious flowering in dates when, after certain field trials, cultures flowered in 3 years — instead of the more normal 15 to 25 years for seedling dates. Similarly, Italy and the United Kingdom have reported unusual and early flowering in apples and cherries during certain tissue culture trials.

In the case of passion fruit, flowering can be achieved under 12 weeks. Sampled tissues include leaves, tendrils, young stems, and flower buds. Regardless of the source of explant material, precocious "de novo" flowering occurred. On some of the larger, leafy plants derived from young stems or nodes flower initiation and, in a few cases, flower development occurred "in vitro".

With the non-woody species, we are on firm ground, with easily demonstrable examples of early flowering from mature tissue available.

These examples may be drawn from the literature and from my own work. For instance, Venus fly trap can be induced to flower within 12 to 15 weeks, "in vitro". *Drosera* and *Byblis* spp. can produce functional flowers in 4 to 6 weeks. Various researchers likewise report "in vitro" flowering of potato, hyacinth, and bouvardia. Bromeliads, grown from seed and multiplied, take a normal time span to flower, but those produced from mature tissue (our lab and several European ones) invariably produce blooms in a significantly shorter time. Potatoes can be induced to produce axillary tubers "in vitro", when tissue is taken from mature, end of the season, plants and can be maintained in this status indefinitely. Such axillary tubers taken out of culture, behave in a completely normal manner in subsequent trials.

It is my belief that any plant that can be cultured, can be induced to exhibit the "maturity factor". Further, any such plant will behave in a completely normal manner when subsequently field-grown. Differences in final height and shape are expected, as such material *will not go through the growth and formative years* of a conventionally propagated plant.

Such differences are a considerable advantage if the end objective for the exercise is to produce flowers, fruit, or seed, in as short as possible time.