SOME LITTLE KNOWN METHODS OF VEGETATIVE PROPAGATION OF SELECTED PLANTS

MARGARET E. MARSTON

Department of Agriculture and Horticulture
University of Nottingham School of Agriculture
Sutton Bonington, Loughborough, England

VEGETATIVE PROPAGATION USING *IN VITRO* CULTURE TECHNIQUES

When the main objective is the rapid multiplication of a selected individual, *in vitro* culture techniques may prove to be the answer. Ever since Morel showed that thousands of potential plants could be produced from one meristem of a *Cymbidium* in a year, it has been the dream of tissue culture workers to repeat 'meristem culture of orchids' with other plants. Plant breeders are particularly interested, as years may elapse between hybridizing and obtaining flowers. When selections are made, selected individuals must be bulked up as rapidly as possible into clones so that cultivar trials may then be carried out. Although plant breeders are only too aware that genetical variation may occur in culture and that precautions must be taken, nevertheless Eucarpia, the European Plant Breeding Association, spent much of its five-day conference, in Leeds, in July, 1973, discussing aseptic methods of vegetative propagation.

The range of material which responds to *in vitro* culture techniques is increasing rapidly. Recently, John Innes Institute has been successful with a number of flower bulbs and corms, including hyacinths, lilies, muscari, narcissi, freesias and gladioli, but so far have failed with tulips. Because 10 years, at least, generally elapse between hybridizing and the commercial introduction of a new bulb selection, attempts to reduce the long period of time are to be welcomed.

Virologists are also keenly interested in aseptic methods of vegetative propagation. Returning to Morel's orchids, it will be remembered that his technique for rapid multiplication followed a chance observation when he was culturing meristems with the aim of obtaining healthy plants from them, because the original plants had cymbidium mosaic. If meristem culture samples, from a flask of young orchid plantlets prove, on testing, to be free of certain viruses then it may be assumed that the remaining plantlets are likewise free. (Should the sample show the presence of these viruses then all plantlets would be destroyed). This is now the aim — to clean up infested plants and at the same time multiply *in vitro*. Much time and labour is saved as testing plants for the presence of viruses may be a long process. Davies (2) at John Innes has developed a technique for rapid multiplication of free-

sias, which he propagates from thin slices of the stem of the inflorescence, and hopes to render infected plants virus-free at the same time. Adams (1) at East Malling Research Station obtained virtually unlimited numbers of healthy strawberries from virus-infected ones, using a meristem culture technique. Greater progress will doubtless be made when we know more about the behaviour of plant viruses in tissue culture. Walkey (5) studied this problem and among other plants was able to free virus-infected cauliflowers from virus by culturing minute portions of the curd.

Is there any place for *in vitro* propagation in the commercial production of plants? For high value plants such as orchids it has already been shown that there is. For difficult-to-propagate plants where, at present, there is no feasible method of vegetative propagation, there well may be. A great deal of research work has already gone into attempts to produce plants of the coconut and oil palm but, so far, woody plants are proving to be exceedingly difficult and few have responded. This summer, there was a T.V. programme about aseptic culture techniques at present being used by a large commercial laboratory in England. This may be the forerunner of specialist concerns which propagate, on factory-lines, high value crops, for sale to nurseries in flasks for growing-on, as if they were young seedlings.

General principles are evolving, although modifications have to be made for different species and even for different cultivars. Plant propagators will be glad to know that tissue culture workers are beginning to realise that the history of the plant, and the plant material selected for propagation, may be important! It is also becoming clear that it is worth studying the plant which one wishes to propagate!

I am now going to describe how we have propagated a few plants by rather unusual methods. I will start with asparagus.

VEGETATIVE PROPAGATION OF ASPARAGUS BY AERIAL SHOOT CLUSTERS

Plants of asparagus (*Asparagus officinalis* L.) are normally produced from seed, but the quantity and quality of edible spears per plant varies considerably. Plant breeding programmes have been handicapped because male and female flowers arise on different plants and there has been no reliable method of vegetative propagation of selected individuals. Division of a plant into several pieces is cumbersome and gives a low rate of multiplication; shoot and root cuttings fail to regenerate.

During the last decade research workers in a number of countries have succeeded in producing plants in vitro, but their subsequent establishment and further growth after transferring

to compost and a normal environment created problems associated with the character of the plantlets and not with lack of green fingers. John S. Aynsley, a research worker at Nottingham, had been doing anatomical studies on such plantlets and had been comparing their growth with that of seedlings. In the course of his work he noticed that sometimes a main shoot of a young seedling carried a small cluster of shoots. Subsequently, he observed that clusters of shoots may develop on shoots of field-grown plants at the end of the season. These clusters are like the aerial part of an asparagus plant; shoots develop one after the other and small buds develop at their base so that a crown is built up. Hence, if these shoot clusters could be induced to produce roots there would be a means of vegetative propagation without resource to aseptic culture techniques.

Quite independently, Yang and Clore (6) of the Washington State University have also observed aerial crown formation and have described the subsequent development of these crowns into apparently normal plants.

Aynsley failed to root the shoot clusters using conventional propagation techniques, bud did so *in vitro* when he used a nutrient medium supplemented with sugars. However, his propagational material was thin and spindly and attempts were then made to induce shoot clusters on asparagus plants grown in pots in a glasshouse and in a growth room. By removing the crown buds of an established plant but retaining some good growing shoots, small shoot clusters did develop on the proximal part of these shoots. Roots formed *in situ* — good rooting was obtained when the plants were laid on their side and the bases of the shoot clusters lightly covered with compost — and so the parent plant looked as if a number of seedlings had been attached to it!

The challenge is now to find a quicker means of inducing the formation of shoot clusters. We would also like to develop a technique for treating clusters like cuttings so that they could be removed from the plant at an early stage.

There should be no risk of genetic variation when asparagus is propagated by shoot clusters, a risk that has to be considered when certain aseptic techniques are used. But whether or not the shoot cluster/aerial crown technique ever becomes an economic proposition, we have a good example of how plant behaviour may be modified by physical manipulation.

PROPAGATION OF HYACINTHS BY SCALING

Since at least the 17th century, the Dutch have propagated hyacinths by "scooping" and by "scoring". Commercially, an artificial propagation method is essential since a hyacinth bulb

only produces about one small offset per year. Present commercial practice entails the critical control of both temperature and of humidity in specially designed bulb chambers. These two techniques are quite unsuitable for use in a plant breeding programme where there is only one bulb available, asscooping and scoring both destroy the parent bulb and the plant breeder has to gamble on ending up with either dozens of small bulbs or none at all!

It is possible, however, to remove a few of the scale leaves which completely surround the bulb, dust the remaining core of the bulb with a fungicide and replant it (4). The bulb suffers a check, but recovers. To facilitate removal of the scale leaves, a circular cut should be made immediately above the basal plate, to a depth of two or three scale leaves depending on the size of the bulb. By then making 4 to 6 cuts of the same depth from the nose of the bulb to the circular cut, a number of "scale pieces" result, in fact they resemble lily scales. The bulb should be left to dry for about an hour before gently teasing off the scale pieces. We have good results by dusting with captan (15% dust) and laying them horizontally in a peat/grit mixture (1:1 by volume). Although the environment is not critical an initial period of some weeks in a warm house (minimum temperature around 16°C) resulted in slightly larger bulbs the first year.

Small bulbs develop, as on lily scales, at the proximal cut surface and within a few months may produce thin and onion-like leaves. When the foliage has died down the original scale piece will be found to have disintegrated, but one or two bulbs should have developed. From then on the small bulbs are treated as if they had been produced by scooping or scoring.

A somewhat modified technique could be used for any of the commercial cultivars where there is no need to retain the parent bulb. The base of the bulb may be sliced off and the remaining upper part quartered. All but the smallest, i.e. innermost, scale pieces are likely to produce one or more small bulbs. The scale pieces may be kept in a cool house which is heated sufficiently to keep out frost. Although fewer bulbs may result than in the conventional techniques of scooping and scoring, the method is quick and does not require any special facilities.

The basal plate should also be inserted as it also may produce a few bulbs!

PROPAGATION OF ROSES BY ROOT CUTTINGS

Over the last few years we have built up a collection of about 40 rose cultivars on their own roots. These have mainly been propagated by one-node cuttings, each consisting of a short piece of stem, one leaf, and its axillary bud (3). This collection

has given us an opportunity to find out whether rose cultivars could be propagated by using root cuttings.

Our first attempt was in January, 1972 with the hybrid tea cultivar 'Fragrant Cloud'. Plants *were* obtained and their flowers proved to be true to type. (Cultivars raised by normal methods of plant breeding should come true, but those arising as 'sports' might not — mutations which are periclinal chimeras are generally not reproduced by root cuttings.) These plants of 'Fragrant Cloud' were grown on in pots. Within months a number of shoots arose from the base of the plant. At first sight this seems to be a good characteristic, but only time will tell whether or not sucker-like shoots subsequently develop from the roots. There is always the possibility that such shoots could be as much of a nuisance as suckers from roses which are budded onto rootstocks.

This last winter we did a small exploratory experiment with 'Prima Ballerina' (H.T.). Root cuttings were inserted in late December 1972, because plants produced early in the new year would then have ahead the whole of a growing season. (Experience with propagation by one-node shoot cuttings has indicated that young shoots may rosette in autumn, unless supplementary light is given, and such checked plants are liable to succumb to attacks of botrytis and mildew.) A number of other cultivars were also propagated in December and in April. Under our conditions much better results were obtained from the earlier date. However, until batches of root cuttings are inserted regularly throughout the year, in a number of different environments, it will not be possible to state whether there is a definite ON/OFF cycle as with root cuttings of red raspberry (*Rubus idaeus* L.).

Many modifications could be made to the techniques which we have tried. Our root cuttings were 5 cm long. All thicknesses from about 3 mm to 15 mm diameter were included, but were not kept separate. Most of the thinnest cuttings did not regenerate, whilst some thick cuttings developed shoots ahead of new roots and many of these shoots withered and died. Our observations suggest that an adventitious shoot develops near to, but not at, the proximal end of the root cutting and the first new adventitious roots develop near to, but not at, the distal end.

In our preliminary work, a greater proportion of root cuttings have regenerated when inserted horizontally than when inserted vertically. Although fewer cuttings may be inserted horizontally than vertically in a given area, a shallower depth of compost is required. It is also quicker to chop a length of root into pieces for horizontal insertion than for vertical insertion, as there is no need to indicate the distal end with a slanting cut.

A technique which is used with raspberry root cuttings and which is worth further investigation with roses is to remove the

developing shoots when a few centimetres long and insert these as shoot cuttings. With the rose cultivars which we have tried such cuttings inserted under mist and given bottom heat rooted readily. The original root cutting if left undisturbed may produce further shoots. If it is the intention to try this modification, root cuttings should be inserted horizontally, as it is much easier to remove shoots from horizontally orientated root cuttings than from vertical ones.

Our root cuttings were inserted in a peat/grit mixture. No growth substances have been tried, but a greater percentage of root cuttings regenerated when they were lightly coated with captan (15% dust) by shaking gently in a polythene bag. The environment does not seem to be critical. In a warm house (minimum temperature around 16°C) those root cuttings given bottom heat at around 20°C regenerated more quickly than those without. Root cuttings in a cool glasshouse which received enough heat to keep out frosts eventually regenerated, but not until a number of months had elapsed. Overall, the percentage of 'Prima Ballerina' root cuttings, made in December 1972, which regenerated in each environment was about 50%, but thick and thin cuttings had been included. When root cuttings of a number of cultivars were inserted in late April their performance was erratic; eight cultivars gave some regeneration whilst 6 failed completely.

Flowering of plants produced from root cuttings inserted in winter is unexpectedly quick. Pot-bound plants in 8.4 cm diameter pots may flower within several months but, although the colour of the flowers may be true, there may be few petals, thus giving the appearance of wild roses. It is probably best to keep plants growing steadily and to remove flower buds and not allow them to flower until late summer.

Experimental work in the future depends on having a supply of roses on their own roots. One great disadvantage in using root cuttings is that the parent plant may be severelychecked—if not destroyed. Nevertheless, if rose bushes from root cuttings prove to be of an acceptable quality, stock plants might be grown specifically for this purpose. Root cuttings could help in year-round production of roses and could provide an indoor winter job requiring relatively unskilled labour. However, this is looking into the future. I must emphasise that this account of propagating roses by root cuttings is based on observational work and that statistical analyses have not been done.

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DISCUSSION

In reply to a question posed by the President, Harold Tukey agreed that in the eastern United States the trend is to the development of specialised commercial laboratories operating on contract for growers rather than individual nurseries attempting to carry out their own work.

Mehlquist enquired as to the effects of nutrition on aerial shoot production but the reply indicated that little was known in this field, asparagus tending to produce its aerial shoots when nutritional and/or environmental conditions were unsatisfactory.

Jim Wells was interested in the comparative performance of roses produced by the different methods of propagation, but the work was at too early a stage to make any reasonable observations. In answer to an enquiry as to why propagate from roots at all the speaker replied — 'Curiosity.' The President asked about cultivar response in the rooting of rose varieties. Dr. Marston, in reply, indicated that although they had not failed with any cultivars there had been a varying response according to cultivar. As to why single leaf bud cuttings had been used, she intimated that usage of plant material did not then exceed that required for budding. Referring to plant development of roses on a rootstock as compared with roses from cuttings she indicated that habit was often different — viz. 'Iceberg' (a white floribunda) was more compact on its own roots.