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Mishael R Marak
Department of Horticulture, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

Rajkumari Asha Devi
Department of Horticulture, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

Corresponding Author:
Mishael R Marak
Department of Horticulture, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

Clonal propagation in horticultural crops

Mishael R Marak and Rajkumari Asha Devi

Abstract
In recent years, the extensive study on clonal propagation, a kind of micro-propagation, has proved to show exemplary results in benefits of crop production. This can be observed in an array of fields ranging from agronomy to horticulture to forestry. Clonal propagation has shown to be of marvellous use in cases where shortage of resources is dealt with. It is a type of asexual reproduction in which genetically similar copies of a cultivar are multiplied. Through clonal propagation, it can thus be concluded that a hassle-free method of *in vitro* micro-propagation of specific clones can be derived. This in turn gives rise to the production of pathogen-free plants, seeds for hybrid production and germplasm preservation. Thus, the process of this method of propagation can be of substantial use in the field of horticulture which may also develop the need for lesser use of the synthetics in on-field practices of cultivation which can help us combat the present day struggle with detrimental soil pollution caused by agricultural cultivation methods.

Keywords: *In vitro*, propagation, horticulture, asexual reproduction, soil pollution, plant bio-technology

Introduction
Clonal propagation, better known as micro-propagation, is now a commercially efficient and practically oriented feature of plant biotechnology. Other plant biotechnologies, such as genetic engineering, production of transgenic plants, etc.) still remain to stay in par with clonal propagation in terms of research and development, practically and in basic levels. Even so, the successful production of transgenic plants relies much, if not specifically, on the ability to regenerate whole plants from those cells, tissues, or organs in which foreign DNA has been inserted and articulated. Therefore, the eventual need for large-scale regeneration of transgenic plants by *in vitro* techniques is obvious for those plant species which are vegetatively propagated (cuttings, grafting, division, and separation; i.e., clonal propagation). It is also, at the same time, required for seed-propagated plants, to produce the first, parent, fertile plants that will be crossed and selected for eventual seed production.
The use of clonal propagation as per records, seem to have begun in the 1940s mainly under experimental, small-scale laboratory conditions. The *in vitro* propagation of some of the important agricultural plants, mainly ornamental crops, by mass production of clonal propagules, became practical in the early 1970s. In recent years, the diversity of various species of plants which can be clonally propagated has significantly increased, and is now practiced on a commercial scale throughout the globe, resulting in over 500 million plants annually; 50-75% of them being flowers and ornamental plants.

Need for Clonal Propagation in Horticulture.
Horticultural crops by default being vegetatively propagated, provides a way to true-to-type clonal plant material. This advantage can in turn be widely exploited for the commercial clonal propagation of such species. In this respect, clonal propagation is tantamount with vegetative method of propagation, the main differences between cuttings and micropropagation being: (1) a very small plant part, usually in the range of a few millimetres or less, is used as the base resource for *in vitro* propagation; (2) the explant is preserved in vials or test tubes, in culture mediums with balanced or optimum pH; (3) aseptic conditions are a must to carry out micro-propagation procedures; and (4) micropropagation typically results in a number of clonal propagules per unit of initial (stock) plant material; many times over that by other means of vegetative propagation, and in a much shorter time. Therefore, a dramatic increase of the propagation coefficient is obtained.

Advantages and Disadvantages

Advantages

Thousands of plants can be produced at a rapid pace by this method depending upon on the rate of multiplication.

The possibility of eliminating viral, bacterial, and fungal contamination during clonal propagation gives rise to the production of disease-free plant material. Contamination of plant material as a source is a primary limiting factor in conventional plant propagation. Clonal propagation and *in vitro* techniques tackle these problems which reduce the risk infection and its negative consequences. This is especially significant for many important agricultural and horticultural crops.

Clonal propagation in horticultural crops itself is drawn to the availability to true-to-type plant material since most of the horticultural crops are traditionally propagated vegetatively. This helps in the production of large stock of true-to-type clonal propagation material.

Planting material (hardened) for clonal propagation can be easily shipped and transported in relatively convenient small spaces as much as 30,000-50,000 *in vitro* plants (in closed vials/test tubes) or 3,000-10,000 plants in a cubic metre area of shipping.

Clonal propagation also gives potential in the bringing of newly bred plants and selections to the market quickly and in large quantities. Selection and breeding of plants have been a lengthy process; with such tissue culture technologies, testing and tracking of new genotypes can be achieved in a matter of months or lesser years. Furthermore, it offers possibilities of patenting cultivars for the needs of the mass market.

Disadvantages

Environmentally induced contamination of plant cultures can arise if proper aseptic conditions are not maintained. This could in turn result in economic losses in commercial clonal propagation.

Poor source of plant selection or incomplete monitoring of clonal propagation methods can result in variations in the plants, genetic or epigenetic.

A significant amount of wastage and failure can arise if *in vitro* plants are not in optimal conditions upon leaving the laboratory after the commencement of the process of acclimatisation.

For the successful trial of the process, large amounts of investments may be needed for the establishment of technology and facilities required for the whole operation.

Establishing an ideal Micropropagation Laboratory

In order out carry out a successful operation of micropropagation, there are certain adherences which need to be followed with specific principles and practices in the laboratory, at all production levels. Media preparation rooms should be easily accessible and provide basic nutrition to all lab products. The space used for plant production must always be aseptic and kept clean with laminar flow hoods, being the central portion of the lab. For growing the plant material, a room with adequate space must be provided so as top facilitate the need to store the plantlets (up to 500,000 plantlets) for as long as necessary till the entire process has completed.

During the conduct of the experiment, it must be assured that each worker maintains definite quality standards, so as to minimise wastage in viable commercial micropropagation. Thus, it is imperative to have well trained personnel which facilitate appropriate bacteriological, virological, chemical, and horticultural testing. They must be well prepared to troubleshoot any sudden complications which may arise in between or during the conduct of the experiment.

Computer-aided information systems are essential for keeping a watch on the long production processes with multiple manipulations and the personnel involved in the work as well. Identification and control of the biological nature of the production system generally necessitates a customised software system.

Clonal propagation is a process which needs to be carried in entirely clean, sterilised, aseptic environmental conditions. This can be assured with the integration of clean room technology, disinfection and sterilisation of all equipment, workspace and resources including the person engaged in the work, surface sterilisation of plant material and culture media, etc.

Environmental conditions with optimum temperature, relative humidity and light are also some of the key parameters which need to be maintained in the micropropagation laboratory. *In vitro* growth for most plants requires adequate temperatures ranging between 22º-27 ºC. Microhumidity in plant growth containers is an important humidity factor and is generally accepted with the relative humidity (RH) ranging between 98-100%. However, in recent studies, it has been concluded that some plants prove to be more effective when RH is 88-94%. Al though there is some photosynthetic activity *in vitro*, plantlets do not entirely depend on photosynthetic carbon fixation for growth. Fluorescent lamps have been the primary light source used for clonal propagation production.

When *in vitro* plant products are ready for distribution, growth containers are shifted to a designated area for sorting, final quality checking and packing. During this, it must be also noted that the containers used for packing, the mode of transport (airplanes, ships, etc.) are properly sanitised so that no contamination occurs during transportation.

Ultimately, it is imperative to keep the plantlets alive till the time of deliverance to the customer, followed by successful acclimatisation and planting out.

Stages Involved in Clonal Propagation

Stage 1: Explant Source and Mother Plants

The better the quality of the plant source (mother), the better success rate of the clonal propagation process. A true-to-type, certified species or cultivar is most preferred for the purpose of mother plant role. These should be free from infections and diseases or with the help of certain *in vitro* procedures, can be made pathogen-free, as approved by routine examinations conducted in compliance with international standards. As such, the mother plant should also be viable and vigorous, i.e., be able to respond to culture conditions which induce intensive cell division and regeneration. For this purpose, selected mother plants are preconditioned by certain horticultural procedures, *viz.* nutrition, irrigation, optimisation of day length, light quality, etc. to render the mother plant fit.

Stage 2: Establishing Explant in Culture Media

When the explant is first derived, its size ranges from 0.1mm to about 1cm. During this stage, the explant can be established in a culture media which stimulates tissue activation and multiplication. Most preferred media for culture in this case is an agar-based media, but apart from this, liquid media can also be used as alternative. The choice of basal media and plant growth regulators varies according to the plant type and

the multiplication method. Treatment of microbial or viral contamination is also to be undertaken for sterile conditions.

Stage 3: Rapid Multiplication
After the successful completion of the above 2 stages, multiplication of masses of tissues are then carried out at a rapid pace. These are then subcultured out onto new culture media which induces propagule proliferation. High amounts of cytokinins usually stimulates continued multiplication of axillary or adventitious shoots, and a higher proportion of auxins is required for callus proliferation (somatic embryogenesis). The combined and balanced adjustment of growth regulators, culture media, and environmental conditions are optimised to attain maximal proliferation of quality plant propagules.

Stage 4: Plantlet Establishment, Elongation and Rooting
After much subculturing and screening, the resulting plantlets are transferred into the final *in vitro* stage. This phase is formulated to inhibit the rapid multiplication and induce the development of fully developed plantlets. These then later can serve as independent propagation units (bulbs, corms, and tubers). During this stage, photosynthesis and other physiological changes required for the autotrophic, ex vitro growth, is also established for further need during acclimatisation. Culture media modifications such as reduction of cytokinin concentrations, increased auxin levels, etc. can help in the achievement of this by simultaneously modifying the environment.

Stage 5: Acclimatisation
The healthy plantlet derived is typically capable of surviving in natural field or protected conditions. In the early stage of acclimatisation, the plantlets are subjected to low-light, high temperatures, and high-humidity conditions induced with fogging. As time passes by, gradual increase in light intensity, ambient regulation of temperature and humidity can be carried out which synchronise with natural conditions. Adequate amounts of rooting hormones can also be applied for initiation of rooting; planting medium must have adequate pH, drainage, and aeration in order to support the plant. If during this process, any *in vitro* plant dies, it must be declared unsuccessful.

Clonal Propagation in some Horticultural Crops
Field and Vegetables Crops
For the past years, most field and vegetable crops have been cultivated and improved with the use of conventional seed technology. At the same time, there are some which have successfully used the technologies of micropropagation for production. These include varieties and cultivars which are genetically engineered to meet the industrial needs and demands. For this, it is imperative to produce virus- or bacteria-free propagation material. Potato (*Solanum tuberosum* L.) is one such vegetable in which most *in vitro* techniques have been successfully applied.

Ornamentals
When clonal propagation was first introduced into the field, it initially invested its ventures in flowers and ornamental plants. Small labs began experimenting with the production of cut flower species, ornamental house plants and foliage crops, and have been the production models for all micropropagation methods since then. The advantages of clonal propagation in the aforesaid species of plants include a low frequency of off-types for most species of flowers and higher uniformity. Availability of planting material in the market throughout the year can also be provided along with significant elimination of diseases in large quantities. An excellent example of the use of clonal propagation in ornamentals can be seen in carnation (*Dianthus caryophyllus*), a very popular cut-flower. During the 1960s and 1970s, the carnation industry experienced high outbreak of diseases resulting in poor commercial production. To combat this, after a period of extensive trials involving various biotechnologies, it was seen that clonal propagation proved to be of highest convenience and efficiency.

Fruits and Plantation Crops
In all the years, fruit trees mostly have not been a high-volume product of clonal propagation. Although, it may be seen that certain micrograft, rooted cuttings, etc. which can be produced for rootstock from the selected, disease-free mother plants with the use of micropropagation has created market potential. Lately, there are specific laboratories which specialise in the clonal propagation of certain fruit species, *viz.* pineapples, palms, and bananas. Since these plants have been cultivated conventionally over the generations, the spread of diseases and low yields are common problems. So, to overcome this hurdle, growers attempt to solve the problems and increase yields with the use micro propagated planting material.

World Production and Distribution
The production of *in vitro* clonal propagation continues to expand on a global scale ever since its unveiling in the field of plant biotechnology. The Netherlands, around the early 1980s, was declared as a pioneering centre for the commercial micropropagation by producing 20 million plants in a year. Towards the end of the decade, the country started producing as much as 50 million plantlets round the year. It was in this decade that the clonal propagation industry began to experience a rapid boost in its popularity with a sharp rise in the number of micropropagation laboratories in the United States, Europe, and Israel. By this time, other parts of the world discovered the establishment of such a biotechnological method capable of high potential being used in developing countries and continents and so production started booming beyond the market demand. By the end of the 1980s, Eastern Europe, Southeast Asia, and South America, with cheaper manpower and personnel made their entries into the clonal propagation market, thereby providing significant competition in the production of orchids, cut flowers, and house plants in particular.
The early 1990s gave way for the European Continent to conduct a survey of professional and commercial laboratories from 21 European countries. Around 500 laboratories (172 commercial) were surveyed and it was found that commercial production values indicated an output of 80-100 million production of plants in the continent, annually. In 1996, Zimmerman conducted a survey of 113 commercial laboratories in the Unites States, from which it was observed that an approximate annual production of plants ranged around 120 million; a certain continuous and rapid growth in the next following decade was predicted on the basis of the survey which was later assertive to the presumption. In 1996, Israel, being a small country, became a very active participant in the micropropagation industry, having around 10

commercial labs with an approximate annual production between 20-25 million plantlets.

The above figures and statistics simply indicate the rise of clonal propagation in the field of biotechnology in developing countries in the aforesaid time span. Till date, the industry seems to be growing and spreading at a rapid pace, making its way to almost all corners of the world. Even in India, the Central Government declared micropropagation an important field of study in research and development (R&D), and the Ministry of Science and Technology supported 150 projects for R&D and field demonstration in nearly 80 different universities around the nation. The strong R&D support from the Ministry of Science and Technology and favourable policies of the Ministries of Commerce, Industries and Agriculture, Government of India, encouraged entrepreneurs and technocrats to set up more than 50 commercial laboratories between 1987 and 1995, with a total installed capacity of 210 million plants per annum. It was seen that from 1986-1989 the targets achieved were 50% of the installed capacity. The percentage increase in production decreased by 50% from 1991 to 1994 and in 1998 there was a negative percentage showing rapid decline. However, between 1999 till date, there has been an average increase of 35% in clonal propagation production per year. This trend resulted into better capacity utilisation of the existing facilities by 2002; and additional facilities are now being set up to increase the total installed capacity in the country to 300 million plants per annum.

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