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MASS PROPAGATION OF FRUIT TREES IN ITALY BY TISSUE CULTURE: PRESENT STATUS AND PERSPECTIVES

F. LORETI and S. MORINI

Institute of Fruit Science
University of Pisa
Via del Borghetto, 80
Pisa, Italy

Abstract. The present status of mass fruit tree micropropagation in Italy is reported. Information is given concerning species, clones, and the amount of trees of various rootstocks and cultivars produced by several laboratories. Methods, materials, and main characteristics of the laboratories, as well as perspectives of this technique are discussed.

In recent years plant tissue culture techniques have been adopted to an increasing extent for the commercial propagation of plants. The success of these techniques is due to important well known advantages over the traditional methods of vegetative propagation.

The tremendous number of studies carried out in a short time on this subject have revealed many improved technical methods and physiological phenomena. Thus, much information is now available on the media and environmental requirements, so the number of species and cultivars currently propagated *in vitro* is continually increasing.

In Italy, as in other countries, tissue culture is a commercially applied practice for propagating a large number of species such as medicinal plants, ornamentals, vegetables, forest and fruit trees. Progress in micropropagation of fruit trees over the past 2 to 3 years has been remarkable.

The satisfactory results obtained by preliminary experience have increased interest in this technique. Today hundreds of thousands of fruit trees are being produced by micropropagation.

The economic and agronomical consequences resulting from the application on a commercial scale of this technique may be of great value for both nursery and fruit-growing activities. In this paper an estimate of the situation concerning fruit tree micropropagation in Italy is made as well as of its perspectives.

Species and number of trees micropropagated. So far, micropropagation has been used mainly to produce apple and peach rotstocks (Fig. 1). This is, firstly, attributed to the great economic importance that these two fruit species have in Italy. Secondly, the need for propagating some difficult-to-root rootstocks, recently introduced in Italy which could overcome problems such as the low resistance of plants grafted on peach seedlings to chlorosis and to water-logging. The number of apple ('M27', 'M26', 'M9', 'MM106', 'MM111') and peach ('INRA GF677', 'INRA GF1869', 'INRA GF43', 'INRA GF655/2') rootstocks produced by tissue culture since the beginning of its application on a commercial scale today is about 2.5 and 8.5 millions, respectively.

Among peach rootstocks, the hybrid peach × almond 'INRA GF677' and Damas 'INRA GF1869', are the most widely propagated ones; 'INRA GF43' and 'INRA GF655/2' are micropropagated to a smaller extent. Clonal rootstocks of plum ('Pixy') and cherry ('Colt') are also giving satisfactory results with this technique. In early 1982, the interest in propagating apple rootstocks increased because of the restrictions imposed by the Italian Ministry of Agriculture relative to the importation of apple trees from other countries: these measures were applied in the attempt to avoid introduction of fireblight into Italy.

Very recently laboratory research showed the possibility of propagating certain apple, pear, peach, plum, cherry and kiwi cultivars (Table 1) on their own roots. Some of them are produced in very small amounts (500 to 2,000 trees) but others, such as 'Armking' and 'Sunred,' 2,000 and 8,000 maiden trees on their own roots were produced, respectively. In 1981, tens of thousands of kiwi plantlets were produced .

Table 1. Fruit tree cultivars of different species propagated by *in vitro* culture.

Species	Cultivars
Peach and nectarine	'Flavorcrest', 'Suncrest', 'Maycrest', 'Sunred', 'Armking', 'Maria Bianca', 'Firebright'.
Apple	'Golden Delicious', 'Golden Delicious B', 'Perleberg 3'.
Pear	'William', 'Decana del Comizio', 'Abate Fetel', 'Conference', 'Kaiser'.
Plum	'Santa Rosa', 'Stanley', 'President', 'Laroda', 'Early Golden', 'Shiro', 'Sorriso di Primavera', 'Ente 707'.
Cherry	'Vittoria', 'Gemella', 'Durone I', 'Durone II'.
Apricot	'S. Castrese', 'Defarges', 'Reale d'Imola', 'Caldesi I'.
Almond	'Ferraudel', 'Tuono', 'S. Caterina'.
Kiwifruit	'Hayward'.

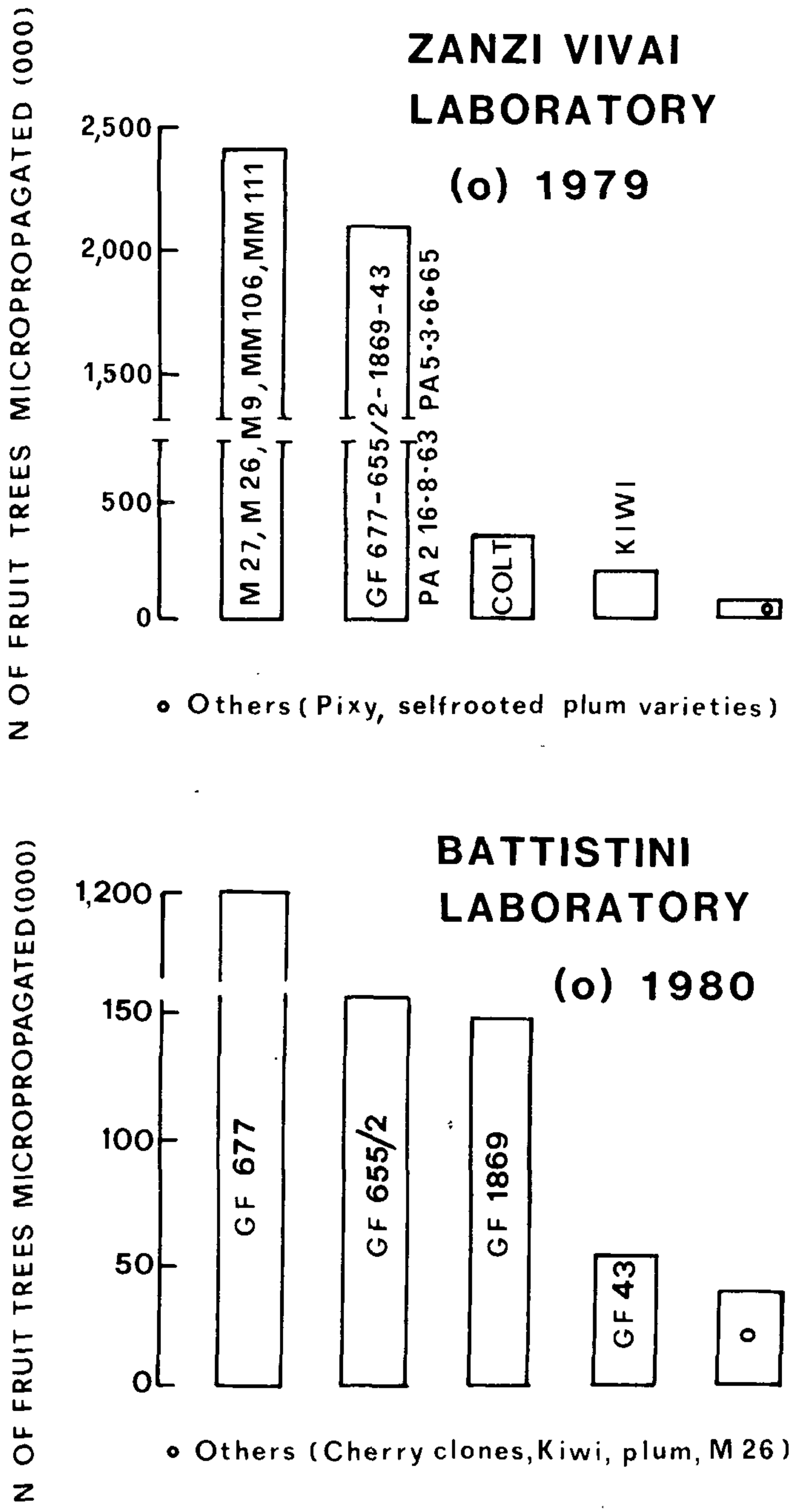
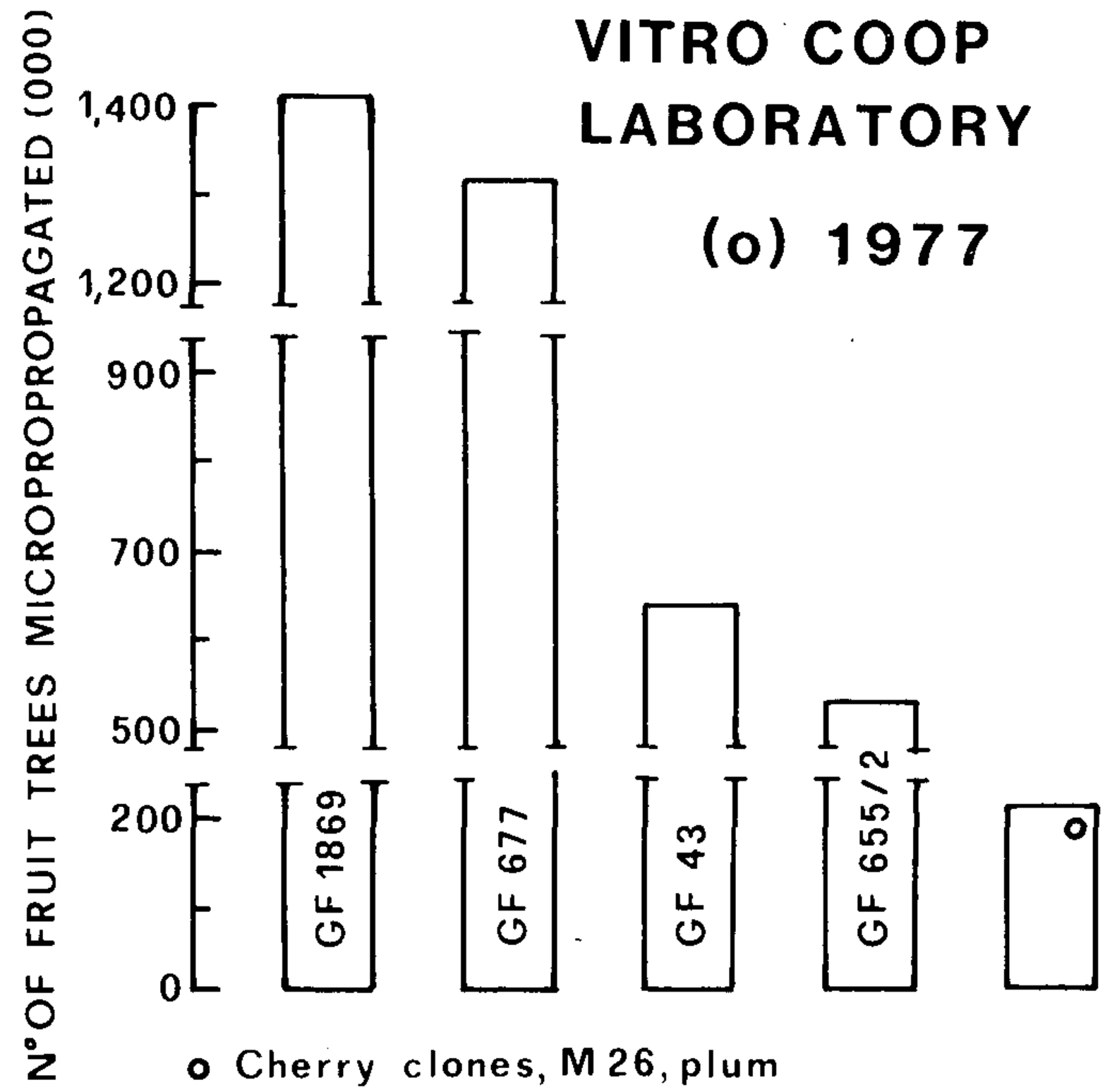


Figure 1. Number of plants produced *in vitro* by different laboratories from the beginning of their activity (0) on commercial scale to today.



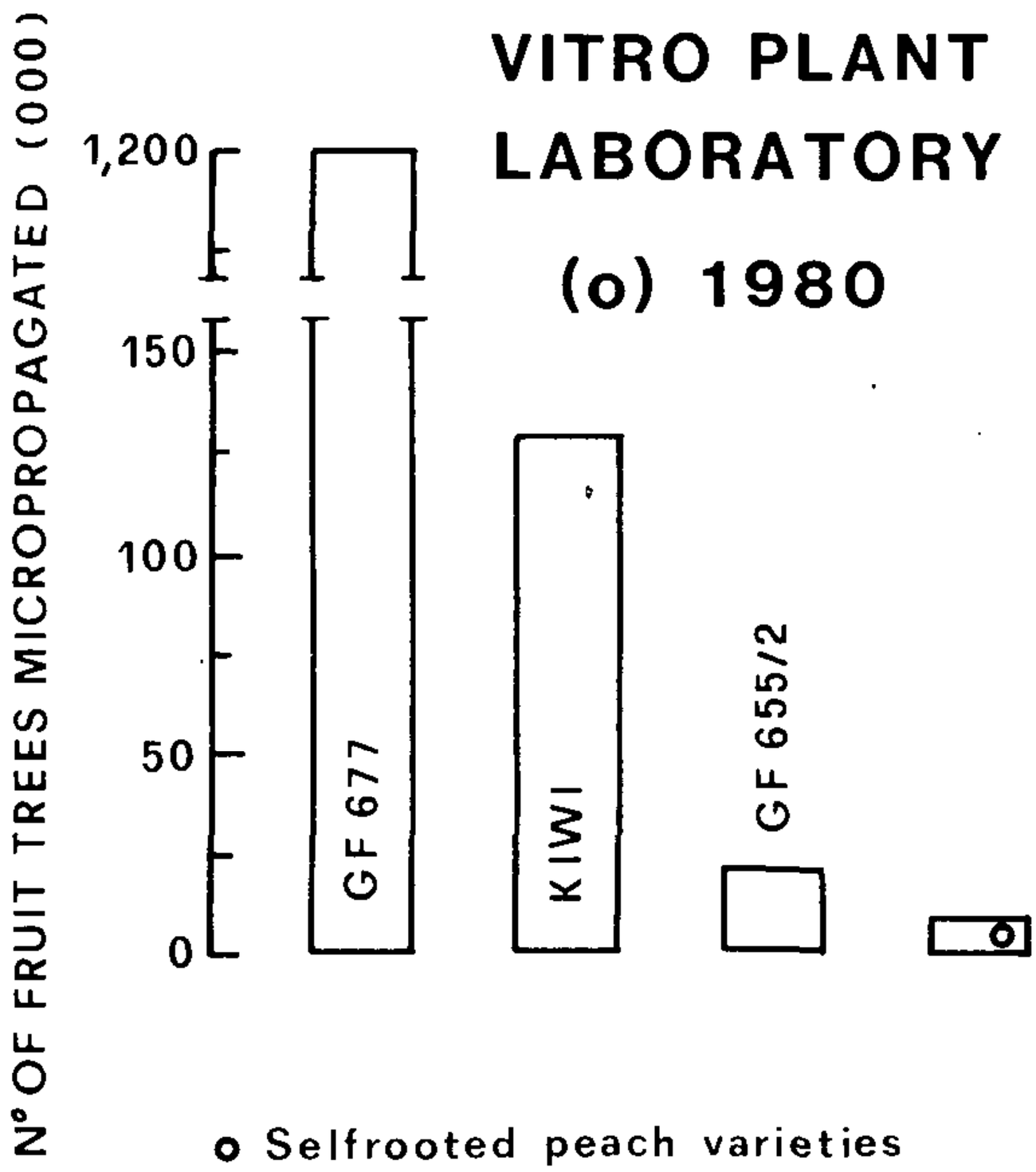


Figure 1. (Continued)

Micropropagation techniques used by different laboratories. In various laboratories, micropropagation is preceded normally by specific experimentation to determine the medium requirements for each species and cultivar. With these trials only a few thousand plants are produced.

In general, whole dormant buds, meristems, and shoot tips have been tried; the latter is preferred because it is easily handled and has given better results. Each laboratory has chosen one or the other method based on these experimental results.

Sterilization was accomplished in different ways depending on the type of material used and based on the experiences of the laboratory. Washing in alcohol or in sodium or calcium hypochlorite solution has been used successfully. In some cases an antibiotic is added to the medium to prevent bacterial growth.

Each laboratory has determined its own balance of the various media components starting with the formulae published in the literature. Media found most suitable for particular clones were determined by accurate experimentation in which the material to be propagated was collected at different times.

The shoot proliferation phase can be carried out in different ways:

1) Rapid multiplication through rapid sub-culturing every 2 to 3 weeks. In this way a large number of shoots are produced but they are usually too short. Under such a situation an elongation phase is necessary to obtain good shoot development in preparation for rooting.

2) Multiplication is obtained at a modest rate by transferring to a fresh medium every 3 to 4 weeks. By this method, longer shoots are produced which are most suitable for handling and are prone to rooting. In this case, the shoots can go onto rooting medium while the bases of the explants can be recultured on a fresh proliferation substrate. The method chosen depends on the requirements of the laboratory or the nursery and on the market.

The various species and clones have differing potentials for shoot proliferation (Table 2); these potentials depend, in part, on the appropriateness of the medium and on biological factors not yet defined clearly.

Subcultures are made every 15 to 30 days; e.g. with 'INRA GF 677' it is possible to subculture the material 14 to 16 times per year. New apices from the mother tree are being explanted each year to initiate new cultures.

Table 2. Potential rates of proliferation of some species starting with 100 sterile apices (based on data from Zanzi Vivai laboratory).

Period of culture (weeks)	A kiwi fruit	B apple	C INRA GF677	D Pixy
0	100	100	100	100
4	200	300	400	500
7	400	900	1,600	2,500
10	800	2,700	6,400	12,500
13	1,600	8,100	25,600	62,500
16	3,200	24,300	102,400	312,500
19	6,400	72,900	409,600	1,562,500

After the proliferation has been completed, an elongation phase on a different medium may be necessary to obtain shoots of desired lengths for rooting. This last phase requires 2 to 4 weeks, depending on species and cultivar. In some cases, such as 'INRA GF 1869' and GF 677', 8 to 12 days are sufficient to reach 80 to 100% rooting. Usually light and temperature conditions during the rooting phase are the same as in the proliferation, although in some laboratories and with some species, light and temperature may be increased.

Acclimation is normally carried out in 30 to 40 days. Some species, e.g. pear, may grow slowly or stop and then resume growth later.

An important aspect of acclimation is the choice of medium, especially the source of peat used. The source of peat and the ratio of peat in the potting mixture may determine the survival and the growth of young plants. Experimental work is continuing to define the optimum chemical and physical attributes of the medium.

The best period to minimize the costs of acclimation is from March to September. Plants produced during winter are refrigerated and acclimated in the spring prior to transplanting to the nursery.

Laboratory organization. Micropropagation of fruit trees is practiced by four commercial laboratories whose production is shown in Figure 1. They are located in Central Italy in the most important area for the cultivation of fruit trees. In each laboratory about 7 to 12 persons are employed of which 2 or 3 prepare media. Besides the facilities which are needed for setting up the *in vitro* culture, each laboratory is supplied with growth rooms and greenhouses for acclimation as shown in Table 3.

These values are, at this time, the maximum capacity of each unit but they are not all designed for fruit tree multiplication. Many vegetable species, ornamental and medicinal

Table 3. Amount of growth room and greenhouse space available at the four micropropagation laboratories.

Laboratories	Illuminated shelving in the growth room (m^2)	Greenhouses for acclimation (m^2)
Zanzi Vivai	345	2,500
Vitro Coop	190	1,600
Vitro Plant	180	1,600
Battistini Vivai	140	2,000

plants as well as forest trees are also micropropagated by these firms. However, fruit trees are the main production which may reach 60 to 80% of total.

Concerning the facilities for eleminating viruses from plants Zanzi Vivai laboratory has been provided since 1962 with heat treatment chambers, glasshouses for indexing, and repository for maintaining the mother plants in a healthy condition. In a short time all the other laboratories will set up these structures; now they utilize virus-free material available at some laboratories belonging to Italian universities or, sometimes purchased from foreign countries.

An important point to be underlined is the collaboration between private laboratories and universities and other Italian and foreign research institutions. Due to this cooperation, the laboratories may have assistance with particular problems of micropropagation and be brought-up-to-date on new developments in this field.

The destination of micropropagated fruit trees is different among the various laboratories. Zanzi Vivai utilize the plants, principally clonal rootstocks, for their own use. Thus the nursery, of which the laboratory is part, utilizes the stocks for their grafted maiden trees of several species. Some plantlets represent material for establishing mother plants (especially apple rootstocks) from which cuttings are collected for conventional propagation. A part of the acclimated plants are sold to nurseries and fruitgrowers or exported to many European, Arabian, and African countries.

Vitro Plant laboratory sells 90% of its production. Normally the plants are sold in an acclimated state but at times, for instance with 'INRA GF677', small lots may be sold at the end of the rooting phase. Last year a few thousand 'INRA GF677', representing about 10% of total production, were exported to Spain.

Battistini laboratory utilizes 20 to 30% of its production for its own nursery activity; 70 to 80% is sold to other nurseries, fruitgrowers and cooperatives. In 1981 about 3 to 4% was exported to other countries.

Finally, Vitro Coop Laboratory, founded by a cooperative

of 4,100 fruitgrowers, supplies principally its members and nurseries. Recently this laboratory has begun to sell nonacclimated plants; in this case the purchasers generally provide the necessary structures to carry out this operation. This allows the laboratories to reduce the risks related to acclimation which represents one of the most expensive phases of micropropagation.

It would be very interesting to talk about micropropagation costs, comparing them with those of conventional methods. Unfortunately many factors make this very difficult to do. One of these factors is the expertise and structures available to the laboratory needed to increase its efficiency.

Another factor is the variability of response of different species. Moreover, the laboratories propagate various species and clones at the same time and it is hard to distinguish the costs for producing a plant of each one. Last, but not least, is the reticence of nurserymen to discuss economic questions.

It is only possible to compare some aspects common with conventional propagation methods. Table 4 shows that micropropagation has a better adaptability from different points of view than conventional propagation.

Table 4. Comparison between micropropagation and conventional methods in relation to some production factors (+ = favourable).

	Micropropagation	Conventional methods
Original stock	+	−
Space necessary	+	−
Time for propagation	+	−
Sanitary conditions	+	−
Unexpected events	−	+
Market response	+	−

Perspectives. The results obtained so far on fruit tree micropropagation can be considered very satisfactory and the perspectives of further spread of this technique are related to the possibility of reducing the production costs and to put on the market trees at a lower price than those conventionally propagated.

The exclusion of a grafting or budding operation and the production of a large amount of self-rooted trees could represent an important step in reducing the production costs. This possibility could only be feasible after the biological and agronomic behaviour of self-rooted trees of the various species and cultivars has been evaluated.

The possibility of propagating by micropropagation rootstocks which are difficult-to-root by traditional methods, could

permit reconsidering some of them which may have superior characteristics and be particularly suitable for specific conditions.

To date the micropropagation laboratories now existing in Italy seem to be sufficient to satisfy the demand of the Italian market for material difficult to root by traditional methods, and also to supply some exports to foreign countries.

If production costs can be conveniently reduced by further perfecting the procedures now used by laboratories of micropropagation, and major distribution and testing of self-rooted trees occurs, it is reasonable to assume that in the near future micropropagation can be more widely adopted for the multiplication of various species of agricultural interest.

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— Vitro Coop, Via Savio 2400— 47023 Cesena (Forli).

— Vitro Plant, SS. 9 Emilia, 5551 — 47023 Budrio — Cesena (Forli).

— Zanzi Vivai — 44040 Fossanova S. Marco — (Ferrara).

PROPAGATION OF 'Mr. S. 2/5' PLUM ROOTSTOCK BY TISSUE CULTURE

F. LORETI, S. MORINI, and C. BARBIERI

Institute of Fruit Science
University of Pisa
Via del Borghetto, 80
Pisa, Italy

Abstract. Observations were made on the behaviour of 'Mr. S. 2/5' rootstock propagated by "in vitro" culture on a modified MS substrate. Shoot tips were collected in February from actively growing shoots kept in a growth chamber. Numerous shoots were produced from the shoot tips during the proliferation phase but many of them did not develop (only a few millimeters in length) even when subjected to the elongation phase. Shoots, elongated on a medium with reduced BAP concentration, gave better results but their rates of rooting were slower and their numbers less than those of shoots elongated on a medium without hormones and with half strength MS nutrients. Plantlet survival was not satisfactory.

During the last two decades the Institute of Fruit Science of the University of Pisa, has carried out clonal selection on some *Prunus* species in an attempt to select rootstocks with better agronomic characteristics and rooting capacity. In this work seedlings of *Prunus insititia*, *P. domestica*, and *P. cerasi-*