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

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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-*

fera were evaluated and promising ones selected. In addition to good rooting as evaluated by trench layering, they were characterized by high vigour, satisfactory growth after transplanting, and graft compatibility with some peach and apricot cultivars.

One of these, 'Mr. S. 2/5', selected among Myrobalan seedlings, has been delivered to nurseries and will be put on the market under a trademark. In order to satisfy demand of nurseries and fruitgrowers it would be very useful to produce quickly a sufficient number of trees to establish mother plants. Research carried out to date on micropropagation shows that this technique provides the methodology to make this possible. In this paper the results of "in vitro" propagation trials of 'Mr. S. 2/5' are reported.

MATERIALS AND METHODS

Cultures were established using actively growing shoots 1 to 2 cm long which were collected in February from 1-year-old shoots. These had been held for a few weeks in a growth chamber in order to promote bud burst and shoot elongation.

Shoot apices were surface sterilized with sodium hypochlorite at concentrations of 0.8% and 1.2% for 15 minutes; a surfactant was added at 0.2%.

The explants were rinsed three times in sterile distilled water and aseptically cut back to obtain 10 to 12 mm length shoot tips. Each was placed on a 10-ml culture medium in glass tubes (2.4 × 16 cm).

To stimulate shoot tip growth, different media were used (7): A) for shoot proliferation; B) for shoot elongation; and C) for shoot rooting. All contained the MS macro and micro elements and were characterized by different levels of growth regulators. The medium A) contained (mg/l): nicotinic acid 0.5; pyridoxine HCl 0.5; glycine 2.0; thiamine HCl 0.1; myo-inositol 100; ascorbic acid 10; NAA 0.01; BAP 0.6; GA_3 0.1; sucrose 30,000. B): the same components as A) but with BAP at 0.1 mg/l and GA_3 at 0.5 mg/l. The effects of B medium were compared to those of R, which did not contain growth regulators but with macro and micro elements reduced by half (5). Medium C) contained the same components as A but without BAP and GA_3; IBA was added at 1 mg/l.

The media were adjusted to pH 5.3 before adding 0.6% of agar; they were then sterilized at 120°C for 20 minutes. IBA was sterilized by passage through a 45μ millipore filter.

Culture room temperature was maintained at 24 to 26°C with 16-hour day and with a light intensity of 3,500 lux.

At three week intervals the cultures were transferred to fresh media. At this time explants which had produced several shoots were subcultured.

Rooted shoots were rinsed under tap water to eliminate any residual agar from the roots. Plantlets were potted in a mixture of soil, perlite, sand, and peat (1:1:1:1), which had been previously sterilized.

Initially the potted plantlets were put in a glass-case where humidity was very high. The case was then gradually opened and the plants were transferred for a few days to a conditioning chamber and afterwards into a greenhouse.

RESULTS

Shoot tips sterilized with 0.8% and 1.2% Na hypochlorite had survival rates of 23% and 37%, respectively. In both trials about 25% of explants were damaged by hypochlorite and did not grow normally. 'Mr. S. 2/5' apices appeared to be more sensitive to hypochlorite than other plum and peach rootstocks (1).

Proliferation of shoot tips started quickly but many of the new shoots appeared to be very short, less than 10 mm (Fig. 1). After about 100 days of growth and after 2 subcultures were made, half of the explants were transferred to medium B and the others to medium R, in order to promote shoot elongation. After 22 days of culture on B or R, the average number of elongated shoots per explant was much higher with medium B than R (Table 1).

Table 1. Average number of shoots elongated in 22 days on two elongation substrates (B and R) and estimate of the average number of shoots which could be produced in 122 days of culturing by an initial shoot tip.

Substrate	Average number of shoots (> 1 cm length) per explant	Average number (x) of shoots (> 1 cm length) per shoot tip
B	8.0	33.1
R	4.4	18.2

(x) These values have been estimated considering the total number of the explants obtained at the end of proliferation phase as cultured on the substrate B or C.

During proliferation and elongation phases some explants produced shoots with abnormal morphology (Fig. 2); shoots axes were thick and leaves were small, light green, and folded downward.

'Mr. S. 2/5' was slower in initiating roots in comparison with other rootstocks cultured on the same medium. The high-

est percentage of rooting (75%) was obtained in 54 days while 'GF 677' required only 20 to 25 days to reach values between 75 to 100%. The abnormal shoots mentioned above rooted as well as the others and formed normal leaves during the rooting phase.

Figure 1. Proliferation phase of 'Mr. S. 2/5' plum rootstock.

Figure 2. Abnormal shoots produced during elongation phase.

Different rooting results were obtained between shoots elongated on media B and R. Shoots, elongated in the absence of hormones, and with macro and micro elements reduced to half concentration (medium R), gave better rooting (Fig. 3). After 30 days in a rooting medium, 55% of shoots elongated in R had rooted while those elongated in B needed 54 days to reach a similar value of rooting.

Each shoot produced about 3 to 5 well-formed roots (Fig. 4). In spite of the good appearance of plantlets, the establishment after transplanting from the test tubes to peat-pots was not always very satisfactory.

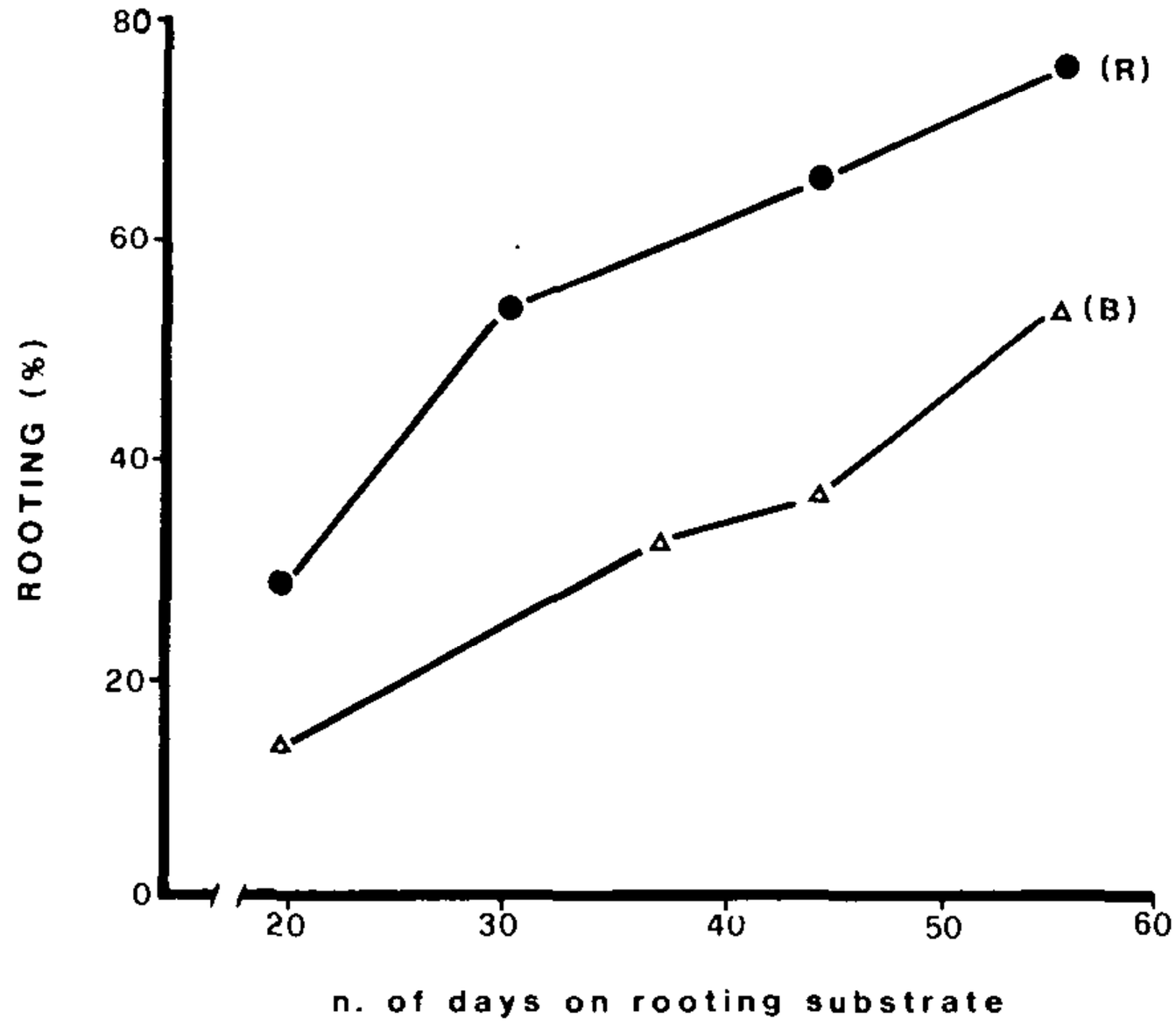

Figure 3. Different rooting percentages observed on medium C of shoots coming from B and R elongation substrates, respectively.

DISCUSSION

The results obtained in this preliminary trial have pointed out the possibility of propagating 'Mr. S. 2/5' by in vitro culture. Nevertheless, further investigations are needed to improve the survival rate of this rootstock to assure mass propagation. A problem to be solved is the shortness of shoots produced during the proliferation phase, which could be attributed to an excess of BAP. The concentration used on this rootstock, even though similar to that used by other experimenters (2,4,6,7), probably stimulated the shoot multiplication to the detriment of shoot elongation. The explants produced a very high number of shoots which grew only to a few millimeters in length, even after 3 weeks of culturing on the elongation medium. The values reported in Table 1 represent the

number of shoots longer than about 1 cm but the shorter ones were even more numerous. From our observations it seems that elongated shoots inhibit the development of others on the same explant. Short shoots present on the explants coming from the elongation phase grew normally after 3 additional weeks when the previously elongated shoots were excised.

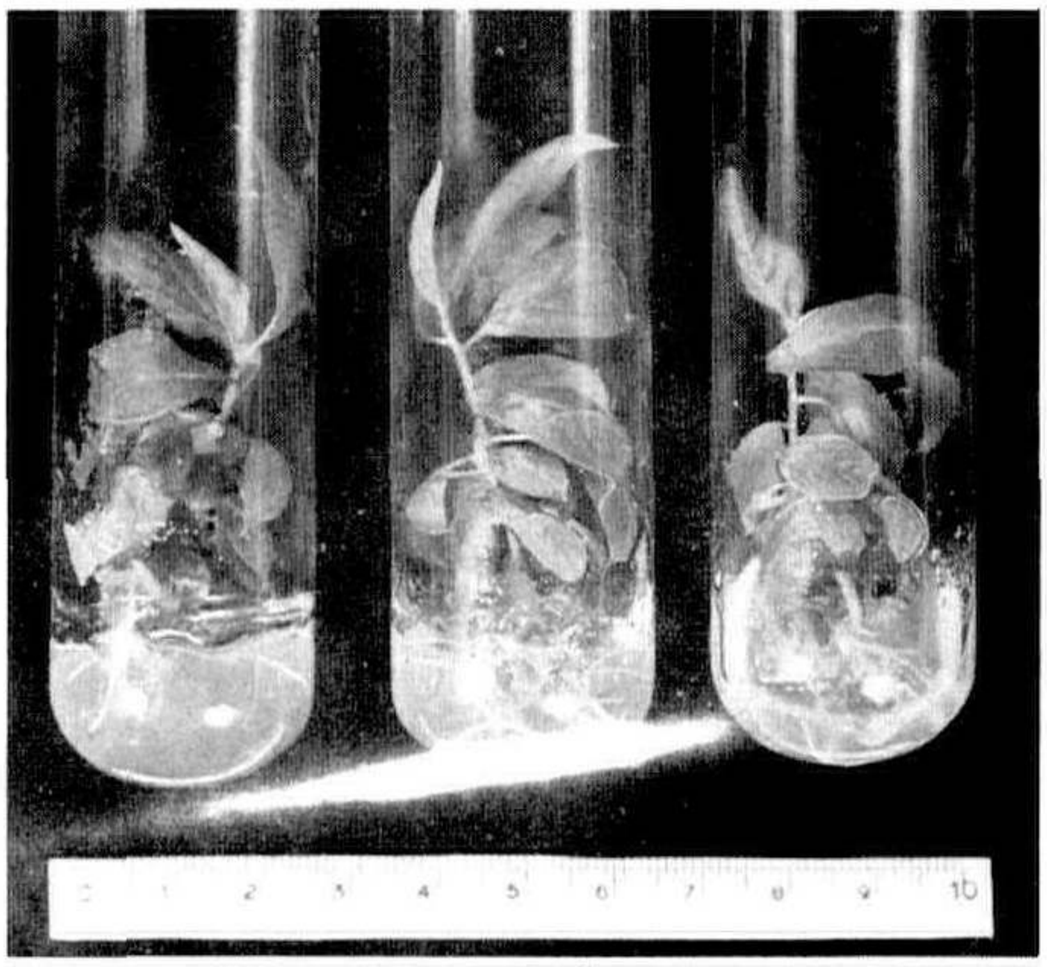

Figure 4. Rooted shoots of 'Mr. S. 2/5' plum rootstock.

Rooting percentage and the length of the rooting period were affected by the components of the elongation media. Thus, the medium without growth regulators and with nutrients reduced to ½ enhanced the rooting effects of IBA and gave more satisfactory results.

The negative response in rooting observed with the shoots coming from substrate B could be related to a residual effect of BAP which was present in the previous elongation medium. Also, in other trials, this growth regulator showed a residual effect wich reduced the rooting capacity of apple shoots (3).

More studies are needed to elucidate the causes of abnormal morphology of new shoots.

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PREPARATION OF PLANTS FOR MICROPROPAGATION

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Several researchers have experienced the frustration in trying to obtain viable, sterile explants. This step, frequently referred to as stage 1 (17), is initially the most important area with which researchers should be concerned. Simply stated, a plant cannot be multiplied effectively if a plant part cannot be properly sterilized. It is the purpose of this paper to reexamine this area and offer possible solutions to these problems.

Whenever possible, the stock plant used for multiplication should be healthy, vigorous, and preferably virus-indexed. If the plant is indexed for certain viruses, care should be taken to prevent reinoculation of that plant. Screening methods have been developed to detect systemic contaminants (2,14).

As in conventional propagation, timing is very important. The physiological state of the plant part will partially determine whether or not the plant will grow, stay dormant, or die.

The environment in which the stock plant is grown is an important consideration. If possible, plants should be grown in a greenhouse. Water should be applied only to the soil so as not to wash contaminants into the axils of the leaves. Plant diseases should be controlled with appropriate fungicides, insecticides, and antibiotics. Systemic pesticides have proven effective. The plants should be grown in a loose, sterile soil mix. This is especially true if attempting to use underground structures as a tissue source. To reduce soil-borne contaminants, plants should be potted to expose those tissues needed for sterilization. When shipping or receiving plant material, it should be sent as quickly as possible and packed in a moist