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SHOOT LOCATION AND COLLECTION TIME EFFECTS ON MERISTEM TIP CULTURE OF SOME APPLE ROOTSTOCKS

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Abstract

The aim of this study was to determine the effects of explant source and collection time on meristem tip culture from M9, MM106, and MM111 clonal apple rootstocks. Meristem tips were collected on 3 different times, from the terminal and lateral shoots of 1-2 year old potted plants. After surface sterilization of the explants, the meristem tips were excised and placed in tubes containing MS ($1/2xNH_4$ NO_3 and K NO_3) medium supplemented with 0.5 mg /l BAP, 0.1 mg/l GA_3 and 0.1 mg/l IBA. The multiplication medium consisted of MS, supplemented with 1 mg/l BAP, 0.5 mg/l GA_3 and 0.1 mg/l IBA. After two consecutive sub culture the shootlets were excised and transferred to the rooting medium containing MS supplemented with 0.5 mg/l IBA.

Shoot formation ratios from the meristem tips were good for the rootstocks, but the collection time and source of the meristem affected these ratios. Higher shoot formation was 95.4% for the M9 from terminal shoots collected on June the 8th, 93.3% for the MM106 from lateral shoots and 81.2% for the MM111 from terminal shoots collected on June the 16th. The optimum explant collection time was the time when the shoots tend to decrease their growth rate. One of the main problems during shootlet formation and multiplication steps was vitrification especially for M9 rootstock. Tissue browning in the explants immediately after establishment in the medium or during early stage of development of the shootlets was also higher especially for MM111 rootstock. General averages of the multiplication coefficients for the rootstocks were as follow: 4.16 for M9, 5.33 for MM106, and 5.74 for MM111. Rooting ability of the shootlets was higher (62.5-90.3%) for MM106, medium (53.6-66.6%) for M9, and low (12.1-40.0%) for MM111. We found that meristem tip culture are difficult with M9 and MM111 but feasible with MM106 rootstock.

Introduction

Clonal apple rootstocks have been widely used in apple growing. Among these M9 (dwarf), MM106 (semi-dwarf), and MM111 (semi-vigorous) have been well known and used in various types of soils and plantation systems. These rootstocks have been propagated by stool bed layering and rooting of hardwood cuttings. The MM106 and MM111 can be rooted easily by hardwood cuttings (Hartmann & Kester, 1983). Some of them such as M9 are propagated with difficulty by conventional methods (Webster & Jones, 1989). As mass clonal propagation is taken into consideration, micro-propagation methods are necessary and some of the apple clonal rootstocks are propagated by these methods (Snir & Erez, 1980). On the other hand, meristem tip culture is necessary with respect to obtain virus free material (Pierik, 1987). As a matter of fact, virus-tested EMLA series of these rootstocks were produced by a joint effort of the East Malling and Long Ashton Research Stations in England (Hartmann & Kester, 1983).

MS basal salt medium or modified MS media have been used widely through the steps of meristem tips, shoot tips or nodal segment cultures of apple rootstocks and the cultivars. However, the types and concentrations of growth regulators added into the basal medium have been critical and different concentrations of cytokinins (BA or BAP), gibberellins (GA_3), or auxins (IAA, NAA, or IBA), have been used for various kinds of

plant materials and various steps of the tissue cultures (Castelli *et al*., 1986; Golośin & Radojević, 1987; Kataeva & Butenko, 1987; Pierik, 1987; Standardi & Micheli, 1988; Webster and Jones, 1989; 1991; Aklan *et al*., 1997; Modgil *et al*., 1999; Dobránszki *et al*., 2000; Radmann *et al*., 2002; Chakrabarty *et al*., 2003).

The cutting season and time of the explants from the stocks are also important factors. Meristem tips can easily be obtained from the actively growing shoot tips (Öztürk, 2004). Therefore, the spring or early summer days might be suitable for this purpose. Active growing season of the trees depends on the climatic conditions. For this reason, the optimum cutting time of the explants needs to be determined for different ecological conditions. In addition, the time to get the explants during day might also be important because CO_2 fixation product varies according to the time of a day in the leaves during photosynthesis and sugar formation is enhanced from the morning through afternoon (Leopold & Kriedemann, 1975). This situation can affect the browning of the explants seen in the woody plants.

The location of the explants e.g., terminal versus lateral shoots, can also affect the growth of the meristems and multiplication capability of the shootlets. It had been stated that isolate position on shoot formation from meristems can be an effective factor (Golaśin & Radojević, 1987).

The aim of the present study was to determine the optimum time to obtain the explants in a season and the position effect of the explant source in meristem tip cultures for the M9, MM106, and MM111 clonal apple rootstocks.

Material and Methods

Plant materials and explant preparations: In this study, meristem tip cultures of 1-2 year old potted grown M9, MM106 and MM111 clonal apple rootstocks were used. Meristem tips were harvested from both terminal and lateral shoots on three different times, 25th of May, 8th and 16th of June (Table 1). The weather temperatures and the mean length of 30 terminal and lateral shoots were recorded separately for each rootstock (Table 1). Fungicide and insecticide were sprayed to the potted plants two days before the collection time. Shoot tips 1-2 cm in lenght were harvested in the morning 07:30-08:30 a.m. These materials were treated with 150 ppm ascorbic acid for about 45 minutes in a glass pot to prevent browning during *In vitro* culture.

Plant materials were washed with 2.5% CuO_4S* $5H_2O$ for 20 minutes, rinsed with 70% ethanol for 30 seconds, treated with 20% commercial bleach (ACE, 5% active chlorine) with tween 20 (1-2 drop) for 15 minutes to sterilize, and then rinsed three times with sterilized distilled water.

Meristem excisions and planting on the culture medium: All work was done in a laminar air flow hood under sterile conditions. Meristems were excised under stereomicroscope (SZ6045TR, Olympus Optical Co. Ltd., Tokyo, Japan) from explants and planted in 2.5x10.0 cm test-tubes singly containing 10 ml of initiation medium. The initiation medium consisted of MS (1/2xNH_4 NO_3 and K NO_3) medium (Murashige & Skoog, 1962) supplemented with 0.5 mg/l BAP + 0.1 mg/l GA_3 + 0.1 mg/l IBA + 0.103 mg/l Murashige and Skoog vitamin powder (Sigma, code M7150) + 30 g/l sucrose + 7 g/l agar (Sigma, code A7921). BAP, GA_3 and IBA concentrations were adjusted to the previous works of Ozturk (2004). The pH of the medium was adjusted to 5.8 prior to agar addition and sterilized in an autoclave for 20 min. Shootlet formation, browning and vitrification ratios were determined in the first step. Twenty five meristem tips were used for all rootstocks, times and shoot types.

Table 1. Collection time, air temperature and the mean length of the shoots on collection dates for the clonal rootstocks.

Material collection period	Collection date and air temperature	Shoot length (cm)			
		Explant source	M9	M106	M111
I. Period	25.05.2005	Terminal	13.19 ± 2.46	11.74 ± 2.93	10.10 ± 3.24
	17ºC	Lateral	11.30 ± 2.56	10.53 ± 2.74	9.19 ± 2.75
II. Period	08.06.2005	Terminal	24.10 ± 4.59	30.33 ± 4.38	25.73 ± 6.14
	19ºC	Lateral	21.63 ± 5.78	26.40 ± 3.71	23.80 ± 4.77
III. Period	16.06.2005	Terminal	24.80 ± 8.29	32.53 ± 7.92	28.33 ± 8.86
	18ºC	Lateral	22.00 ± 5.80	28.67 ± 8.38	28.44 ± 6.28

Multiplication: When the new shootlets were about 1-2 cm in lenght in the vial, they were excised and subcultured every 6 weeks in order to multiplicate the shootlets. The multiplication medium consisted of Murashige & Skoog (1962), supplemented with as above except 1 mg/l BAP + 0.5 mg/l GA_3. The rate of shootlet multiplication was determined by counting the number of new shootlets produced per explant. Generally two subcultures were performed for each rootstock.

Rooting: When the shootlets were about 2-3 cm in length, they were excised and transferred to the rooting medium contained MS medium (Murashige & Skoog, 1962) supplemented with above except 0.5 mg/l IBA. For rooting, shootlets placed in a glass pot and they were transferred into a dark cabinet, 23ºC ± 2ºC for the first 7 days, and then to the normal conditions in the culture room under 16/8 h light regime. But the basal portions of the tubes were covered with aluminum folios to prevent the light penetration. The rate of rooting was determined.

Culture conditions: All the cultures were performed in a growth chamber with a temperature adjusted to 23±2 ºC, and 16 h light and 8 h dark photoperiod provided by warm-white fluorescent lamps given the light intensity about 3500-4000 lux.

Results and Discussion

Shootlet formation: Shootlet formation percentages from the meristem tips varied among the rootstocks and collection times. The highest ratios obtained were as followes: 95.4% for the M9 rootstock from the terminal shoots on June the 8th; 93.3% for the MM106 rootstock from lateral shoots on June the 16th, and 81.2% for the MM111 rootstock from terminal shoots on June the 16th (Fig. 1). This variation may come from the growth characteristics of the rootstocks. Namely, the M9 rootstock showed fast growing during the first collection time and the growth rate decreased thereafter. However, rapid growth tendency decreased in the third period of collection time for the MM106 and MM111 rootstocks (Fig. 2). So it can be stated that the optimum explant collection time was the time when the shoots tend to decrease their growth rate. Some differences were observed between the terminal and lateral shoots in this respect, probably due to the growth characteristics (Fig. 2) and hormonal balance within the shoots.

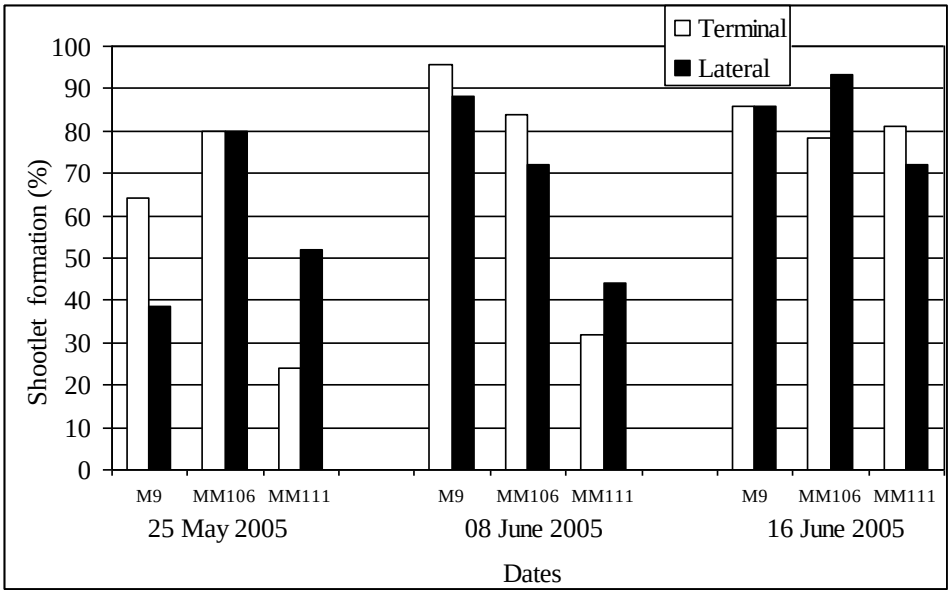

Fig. 1. Shootlet formation ratios of the meristem explants from lateral and terminal shoots for different collection dates.

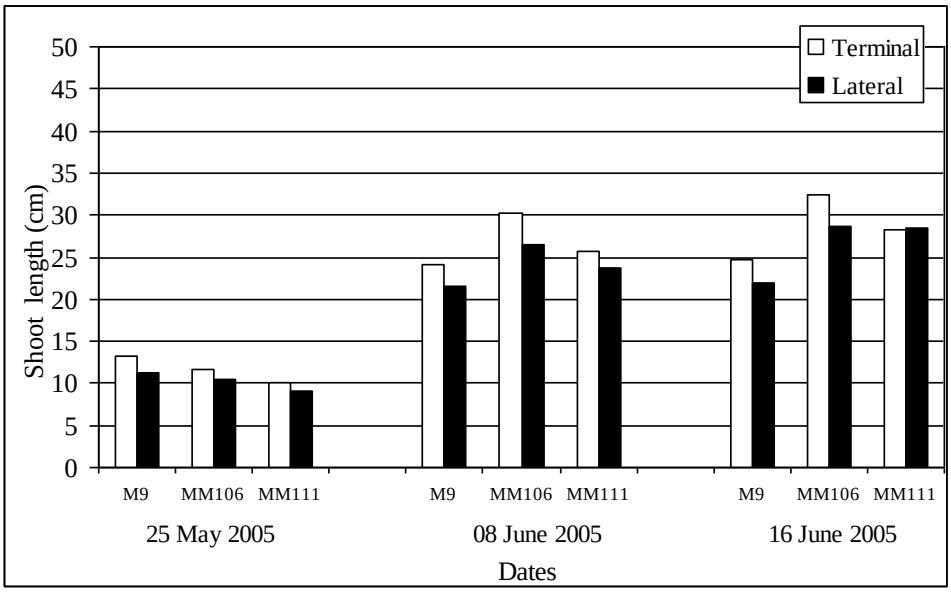

Fig. 2. Mean shoot length of the rootstocks for various collection dates.

Vitrification and browning were observed during the shootlet growing. The ratios of these phenomena are given in Figs. 3 and 4. Vitrification was observed mainly for the M9 rootstock (Fig. 3). However browning (%) were the highest for the MM111 among the rootstocks for all the explant collection times (Fig. 4). So the number of the shootlets brought to the multiplication step decreased for this reason. One of the main problems during shootlet formation and also in the multiplication steps was vitrification especially for the M9 rootstock. The shootlets vitrified were about half of the total (31.2-45.4%) after shootlets formation in this rootstock. This phenomenon was observed for various species and clones (Leonhardt & Kandeler, 1987; Pâques & Boxus, 1987a,b; Standardi & Micheli, 1988; Aklan *et al*., 1997). Pâques & Boxus (1987b) reported that vitrification was related to the nutrient medium composition. The researchers recommended some different procedures to overcome this problem, such as using high agar concentration, reduced humidity and good gas exchange in the medium (Leonhardt & Kandeler, 1987); using shootlets with 4-6 leaves at the beginning of subculture and using solid medium (Standardi & Micheli, 1988);adding phloroglucinol (81 mg/l) in the medium (Aklan *et al*., 1997); small decrease in the relative humidity *In vitro* to increase the plantlet respiration (Gribble, 1999); temporary immersion (ebb and flood system) culture (Chakrabarty *et al*., 2003); increased agar concentration to 8 g/l in the culture medium (Öztürk, 2004). However genotype of the explants source may also be an important factor in this respect.

Tissue browning in the explants immediately after establishment in the medium or during early stage of development of the shootlets was also higher especially for the MM111 rootstock (Fig. 3). Hu and Wang (1983) recommended to treat explants with ascorbic acid to prevent this phenomenon. Probably increasing the ascorbic acid concentration at the beginning of treatments can prevent the browning. Collection time of the explants and BAP level can also affect the browning ratio. Optimum BAP concentrations were 0.5 - 1.0 g/l in this respect (Özturk, 2004).

Multiplication: Two subcultures were performed for each rootstock. Mean values of the multiplication coefficients ranged from 3.75 to 8.08 (Fig. 5). The highest value (8.08) was obtained from the shootlets of the MM111 rootstock originally coming from the terminal shoot meristems collected on June the 8th; and the lowest (3.75) was obtained from the shootlets of the M9 rootstock originally coming from terminal shoot meristems collected on May the 25th. Good results were obtained from the explants of terminal shoots for the MM106 and MM111 rootstocks except the last collection time of MM106. There was no stable tendency observed for the M9 in this respect (Fig. 5). Multiplication coefficient values were fairly higher than the findings of some researchers (Aklan *et al*., 1997; Öztürk, 2004). No clear relationship was observed between the explant collection time and the multiplication rates.

Vitrification problem was also a serious problem at this stage for the M9 rootstock, for both terminal and lateral shoots'shootlets and the ratio increased up to 85.3% level (Fig. 6). Vitrification level decreased for the MM111 rootstock in the shootlets originally coming from the lateral shoot' meristems collected on June the 16th. The MM106 rootstock showed the least vitrification ratios of 0.0-11.5% (Fig. 6), averaging was only 3.15%.

Rooting: Rooting ability of the shootlets was higher (62.5-90.3%) for the MM106 rootstock; medium (53.6-66.6 %) for the M9 rootstock; and low (12.1-40.0%) for the MM111 rootstock (Fig. 7). Rooting ratios of the shootlets were higher for the MM106 rootstock than the other rootstocks. This result was parallel to the findings of some other researchers (Aklan *et al*., 1997; Öztürk, 2004).

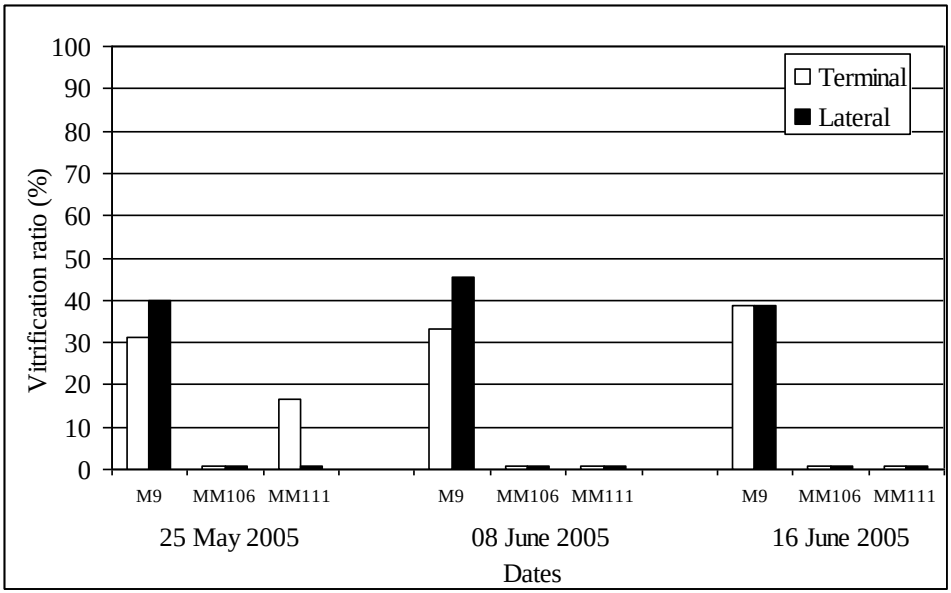

Fig. 3. Vitrification ratios during growth of the shootlets from the meristems.

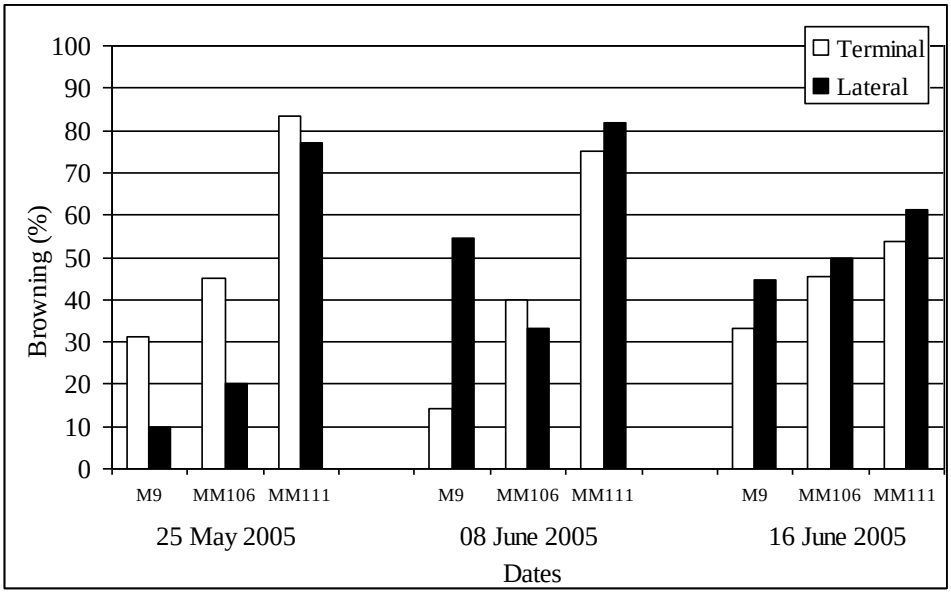

Fig. 4. Browning ratios during growth of the shootlets from the meristems.

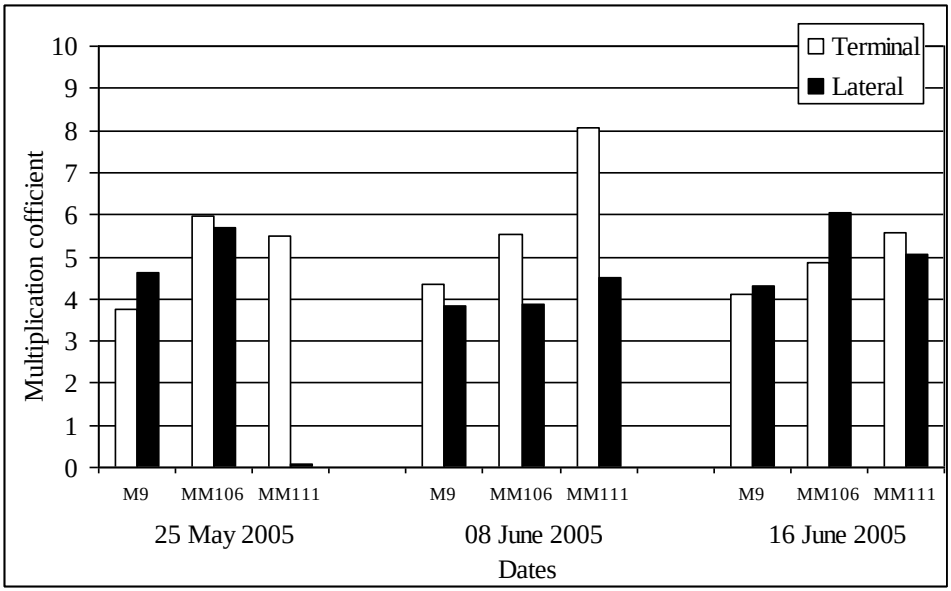

Fig. 5. Variations in mean multiplication coefficient values of shootlets coming from the explants of terminal and lateral shoots for different dates.

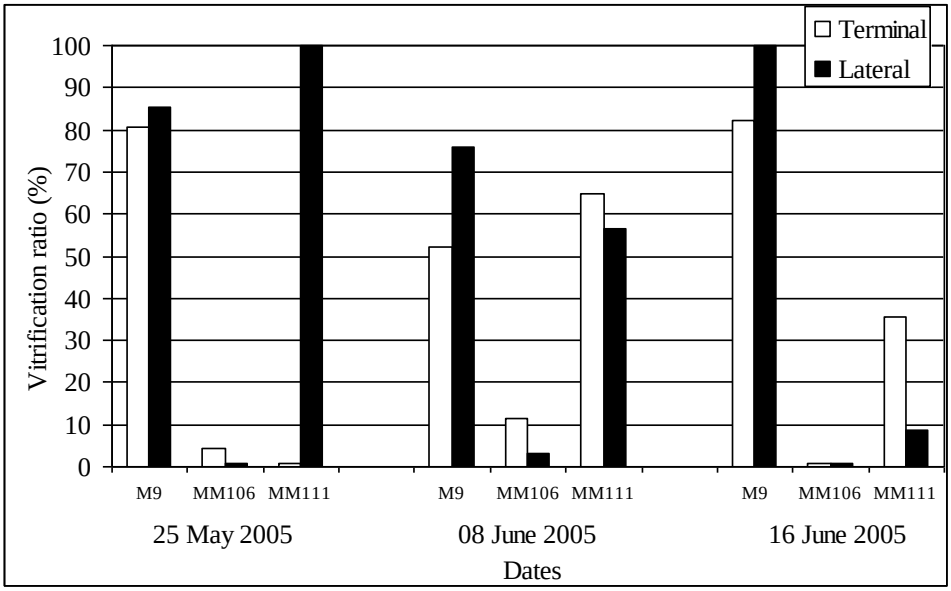

Fig. 6. Vitrification ratios of shootlets during multiplication step.

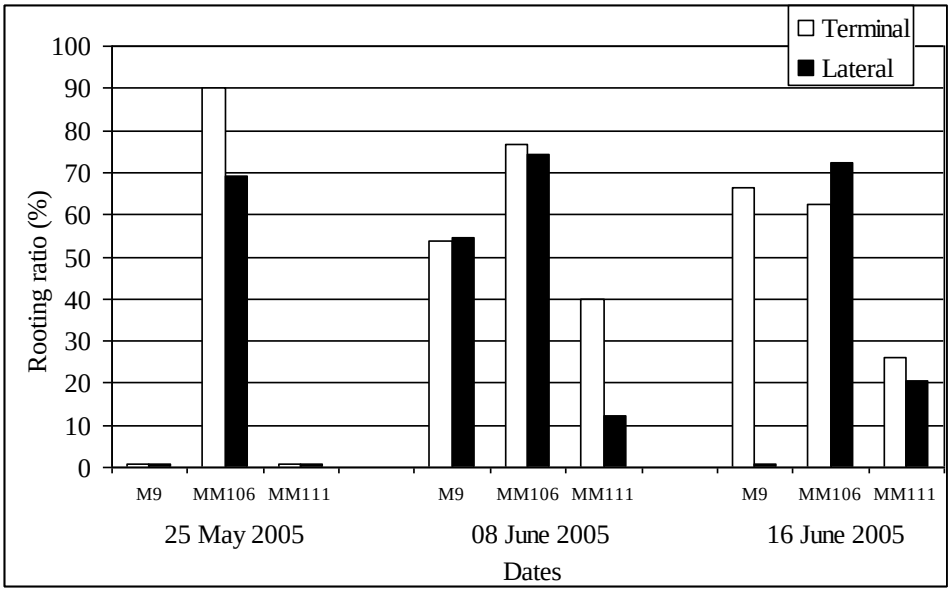

Fig. 7. Rooting ratios of the shootlets coming from the explants of terminal and lateral shoots for different collection dates.

The shootlets coming from the meristems of the terminal shoots showed higher rooting tendency in some cases (Fig. 7). Collection time of the meristems partially influenced the rooting ratio and the first two gave higher results for the MM106 rootstock. The tendency of other rootstocks was not clear.

In conclusion it can be stated that micro-propagation with meristem tip culture is difficult for the M9 as stated Webster and Jones (1991) and for the MM111, but it was feasible for the MM106 rootstock.

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