Micropropagation enhances in vitro establishment and multiplication of new cultures from field grown plants of 'Adesoto 101' (*Prunus insititia*) rootstock

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Abstract

The establishment of new in vitro cultures is often a difficult task due to low growth of initial explants. The explant has to adapt to the new nutritional and environmental conditions, in addition to the surface-disinfection process. Since the explant origin plays an important role, in this work, the effect of the origin of the explants (micropropagated or conventionally propagated plants) in both establishment and multiplication of the in vitro cultures has been studied. While Adesoto 101 (*Prunus insititia*) shows interesting features as a rootstock, it has a poor rooting ability by cuttings, what makes micropropagation the method of choice. Explants (axillary buds), taken from plants previously micropropagated, were compared to those from conventionally propagated plants. Field-grown and frame-grown plants were used as explant source. Three culture media, widely used for fruit trees, were compared for both establishment and multiplication of the cultures: Murashige y Skoog (1962), Woody Plant Medium (Lloyd and McCown, 1981) and Quoirin and Lepoivre (1977). These media were supplemented with sucrose (3%) and Difco-Bacto Agar (0.7 %). Best results during establishment were obtained with explants from micropropagated plants grown either in the frame or in the field. Plants were severely pruned to form hedges, however field growing affected negatively the establishment of new in vitro cultures compared to frame growing. The multiplication rate of new cultures was positively affected by previous in vitro micropropagation of mother plants, while the multiplication rate of cultures originated from plants propagated by cuttings decreased noticeably. Culture medium composition had a different effect depending on the micropropagation phase. While WP gave the best results during establishment, MS, with a higher mineral salts concentration, induced the highest multiplication rate.

INTRODUCTION

Propagation of woody plants is often difficult by conventional means, however, micropropagation has solved in part this problem, since plants can acquire higher rooting capabilities after continuous subculturing in vitro (Hammat and Grant, 1993; Grant and Hammat, 1999). This sort of 'rejuvenation' can influence other aspects of plant propagation as the ability to initiate new healthy-growing in vitro cultures during the establishment, where the origin of the mother plant affects the success of the initiation of the culture. In a previous work (Andreu and Marín, 1994) it was shown that micropropagated plants were a better source of explants than plants propagated by conventional means. In this work, besides the effect of the type of propagation and the

composition of the culture media, the effect of the environment where the donor plants were grown was studied both in the establishment and in the multiplication phase of new in vitro cultures.

MATERIALS AND METHODS

'Adesoto 101' (*Prunus insititia*), an interesting rootstock but difficult to propagate by cuttings, has been used in this work. Explants (axillary buds) were taken in spring from plants propagated both by cuttings or by micropropagation, and grown in two different environments, in a heavy and calcareous soil (field-grown plants), or in a peat-sand substrate (1:1) (frame-grown plants). Trees were severely pruned every winter. After washing in running tap water, the explants were surface disinfected with $HgCl_2$ (0.05%) for 15 min and rinsed 3 times in sterile distilled water, and then placed in tubes with 15 ml of culture medium of three different compositions commonly used with fruit tree species: Murashige and Skoog (MS) (1962), Woody Plant Medium (WP) (Lloyd and McCown, 1981), and Quoirin and Lepoivre (LP) (1977), all three media were supplemented with IBA (0.5 µM), BA (5 µM), sucrose (3 %), and Difco-Bacto Agar (0.7 %). pH was adjusted to 5.6 before autoclaving. Explants were cultured at 22º C under a photoperiod of 16h of cool-white fluorescent light (35 $\mu E \cdot m^{-2} \cdot s^{-1}$) and transplanted to fresh medium at 4 week interval. The number of buds per treatment was 24, and the whole experiment was repeated three times.

Different stages were recorded during the initial phase of growth in vitro: C0, without any growth of the bud; C1, buds swelling; C2, leaves were visible; C3, leaves were completely formed; C4, multiplication started. New shoots were cut off and placed again in the same medium, and the number of shots was scored.

RESULTS

Establishment

New in vitro cultures initiated from buds taken from previously micropropagated plants showed better growth than from cutting-derived plants, for both field- and frame-grown plants. While 70 % of the explants taken from micropropagated plants reached a C3 stage, only 40 % of the explants taken from cuttings-derived plants reached this stage (Figure 1). Moreover, some of the explants from cuttings-derived plants declined after a prolonged culture becoming yellowish and stopping growth. Media composition had an important effect on the initial growth, being WP the most adequate for this phase of culture for both field- and frame-grown plants. Buds taken from frame-grown micropropagated plants and cultured on WP medium showed an earlier development since 29 % of the explants were in C3 stage after 14 days of culture, while only 14% or 11 % when cutivated in MS or LP media respectively. On the other hand, explants from field-grown plants initiated growth later than from frame-grown plants. Thus, after 14 days of culture, no cutting-derived explants reached the C3 stage, while only few micropropagated-derived explants did.

Multiplication

Cultures obtained from micropropagated plants produced much more new shoots than those from cutting derived plants (Figure 2). Composition of the culture medium had a big influence in multiplication, mainly for cultures derived from micropropagated plants, where MS yielded the highest number of new shoots; being LP the medium with

lower number of new shoots. However, the environment of the donor plants had no clear effect on multiplication.

DISCUSSION

Propagation type

Data shown here indicate that the type of propagation of the donor plants had a great influence in the establishment and multiplication of new in vitro cultures, derived from explants (axillary buds) taken from Adesoto 101 trees grown under different environments. Higher percentages of growing buds were recorded when the cultures were initiated from explants taken from micropropagated plants than from cutting-derived plants. Moreover, the explants from micropropagated plants developed into shoots earlier than from cutting-derived plants. These facts might be related with the acquisition of the rooting capability that some difficult-to-root woody plants exhibited after a period of in vitro culture (Hammat and Grant, 1993; Howard et al, 1989; Jones y Webster, 1989) what have been attributed to 'rejuvenation'. Explants taken from juvenile plants performed better than from mature plants (Jones, 1985). In addition, the acclimatization of micropropagated plants has been reported to play a role on the restoration of rooting capability in olive trees (Vidoy Mercado et al, 2001), and micropropagated plants could have received here a similar influence.

Environment

In addition, the environment where the donor plants were grown played an important role on the establishment of new cultures. The explants taken from frame grown plants had a better behaviour during the establishment of new cultures in vitro than those from field grown plants. Field grown plants might have suffered some abiotic stresses that have been avoided in a frame environment, where substrate composition and watering were easily controlled, and that could have affected the physiological state of the plants. It has been described that pre-conditioning of mother plants, growing them in pots in the greenhouse, is very important to obtain success during the establishment of new cultures (Debergh and Maene, 1981). However, this environment effect was not clearly observed during the multiplication phase that started after repeated subculturing in vitro, what could fade this effect out.

Culture medium composition

Composition of the culture medium affected initial growth and multiplication in a different way. While WP was better for the establishment and initial growth of new cultures, MS produced, in general, more shoots, mainly for cultures derived from the best combination of treatments, i.e., micropropagated plants grown under frame conditions. Data shown here suggest that it is advisable to use a different culture medium for each culture phase to obtain optimum results. It would be necessary to set a particular culture media combination for each culture. In our case, the use of explants taken from micropropagated plants grown under frame conditions, and cultured in vitro in a WP medium during the establishment of the culture and in MS during multiplication gave the best results.

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Figures

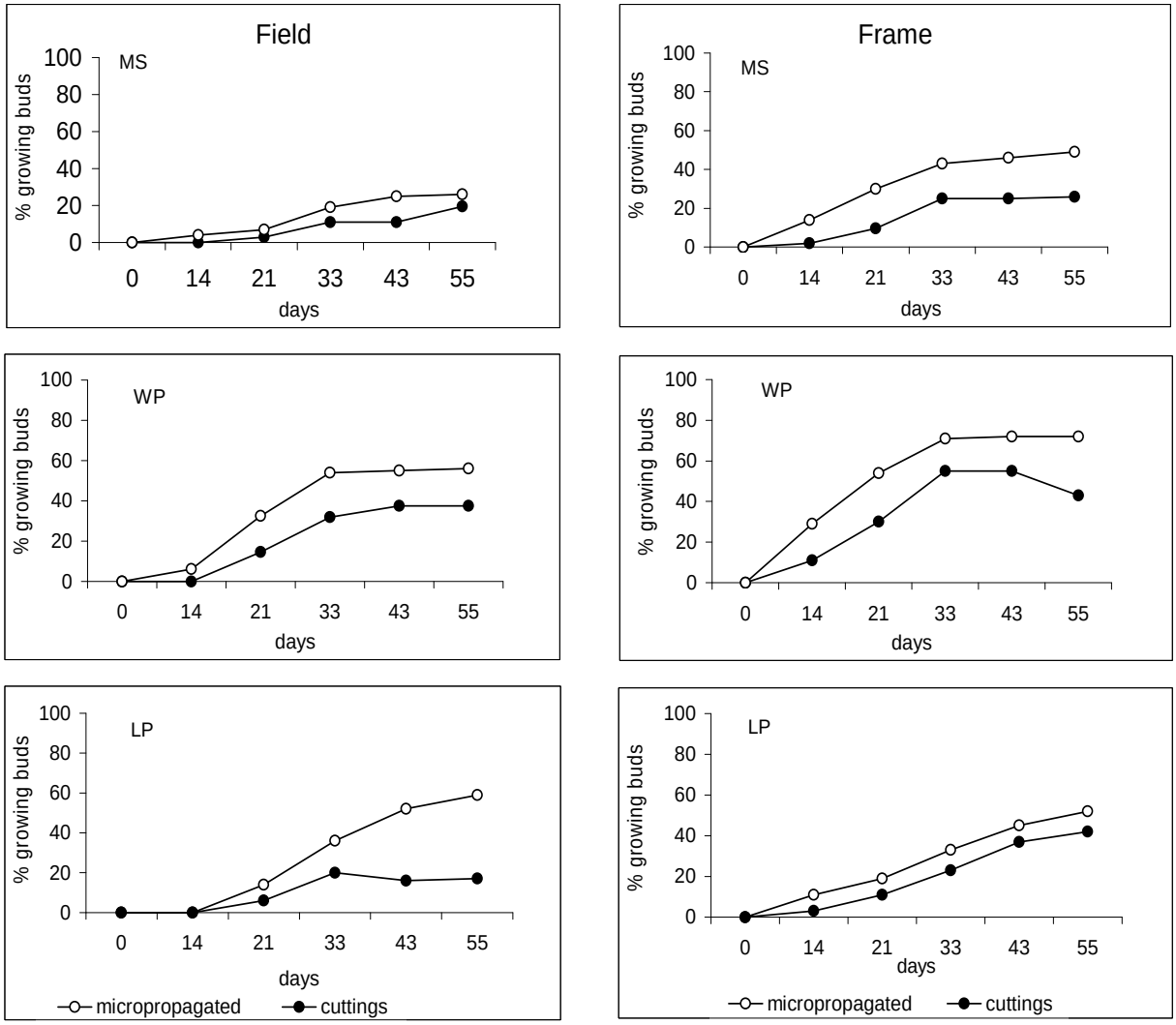

Fig. 1. Effect of different establishment culture media on the percentage of explants in C3 stage (leaves completely formed) of Adesoto 101 taken from micropropagated- and cutting-derived plants grown in both field or frame conditions.

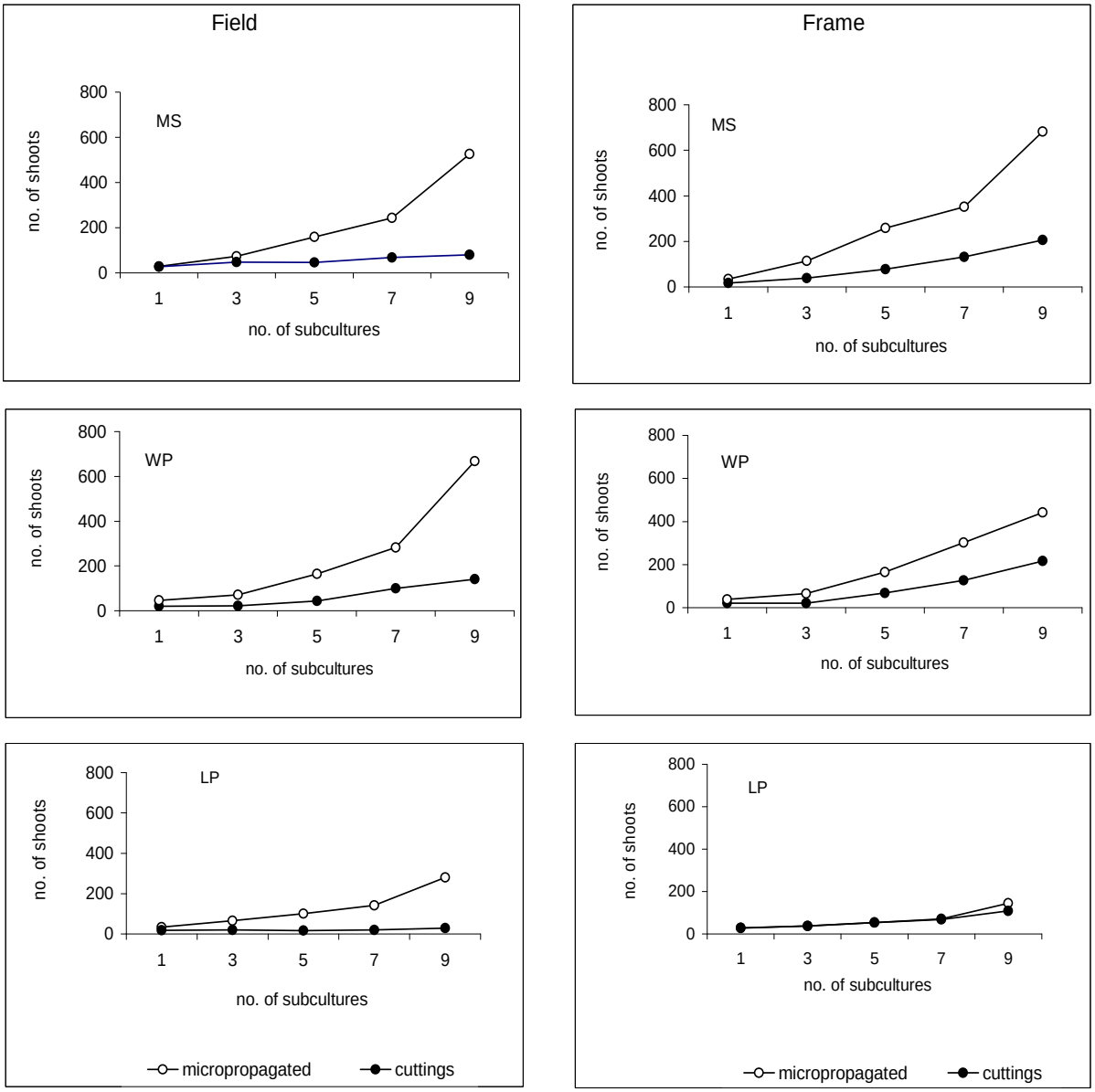

Fig. 2. Effect of different culture media on the number of shoots of Adesoto 101 obtained in multiplication cultures from micropropagated- and cutting-derived plants grown in both field or frame conditions.