ACCLIMATIZATION OF THE MICROPROPAGATED CHERRY ROOTSTOCK 'MASTO DE MONTAÑANA' (PRUNUS CERASUS L.)

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Abstract

'Masto de Montañaña', despite being an interesting cherry rootstock, is little used because of its difficult propagation by cuttings. Micropropagation has been attempted, but one of the major problems has been acclimatization. High agar concentration in the rooting medium increased survival rate while a root developing medium, after a root induction period, did not. The highest survival rate was obtained with brief exposures of rooted plants to low relative humidity in the acclimatization process. These observations are discussed in terms of the stimulative mechanisms required so that plants may adapt to the new environmental conditions.

1. Introduction

'Masto de Montañaña' (*P r u n u s c e r a s u s* L.) is a local cherry rootstock that reduces vigour and is adapted to calcareous soils which are unsuitable for *P r u n u s m a h a l e b* (Herrero, 1970; Cambra, 1979). Despite these interesting features its use is reduced because of its difficult propagation by cuttings, so micropropagation has been attempted.

P r u n u s c e r a s u s has been micropropagated by several authors (Rosati et al., 1979; Ponchia et al., 1980; Cossio, 1981; Cossio et al., 1981; Snir, 1983) with different results. In a previous work (Marín et al., 1983) one of the major problems was the poor survival rate in the acclimatization of the micropropagated plants. The reason of a poor acclimatization has been explained in terms of desiccation (Sutter et al., 1979; Brainerd et al., 1981). However, prevention of desiccation does not always lead to higher survival (Marín et al., 1983; Ponchia et al., 1980). Alternatively plants may not be able to survive in ex vitro conditions because of their physiological stage. This could be overcome by stimulating their fitness to ex vitro conditions during acclimatization.

This work describes the effect of different rooting media on acclimatization as well as the effect of brief exposures to low relative humidity in the acclimatization process.

2. Material and methods

The procedures followed to establish tissue culture were described in a previous work (Marín et al., 1983).

2.1. Culture media

The multiplication medium is based in Zuchercelli (1979) and Jones

et al. (1977). It is composed of the mineral salts of Murashige and Skoog (1962) but with FeNa_2EDTA (37 mg/l) and with the following organic components (mg/l): m-inositol (100), thiamine-HCl (0.4), pyridoxine-HCl (0.5), nicotinic acid (0.5), ascorbic acid (10), glycine (2), benzylaminopurine (BA) (0.7), indole-3-butyric acid (IBA) (0.1), gibberellic acid (GA_3) (0.1), sucrose (30 000) and bacto-agar (6 000). The pH was adjusted to 5.2 with 0.5 N KOH before adding agar. Culture medium was dispensed in 0.5 l jars (100 ml/jar) and autoclaved at 120°C for 15 min. Before rooting, the explants were placed in a shoot elongation medium with the same composition except for BA that was reduced to 0.1 mg/l. In the rooting medium the concentration of mineral salts were reduced to half strength. Ascorbic acid, BA and GA_3 were omitted and IBA was increased up to 1 mg/l.

2.2. Effect of agar concentration

Three agar concentrations (6, 10 and 14 g/l) were used to see their effect on the survival rate. After rooting, plants were transferred to a tray with peat/perlite (1:1) covered by a plastic tent in a culture chamber under 10 000 lx, 25°C and 16 h photoperiod.

2.3. Effect of a root developing medium

After root induction in the rooting medium, shoots were transferred to a similar medium but without IBA. The root induction periods were 8, 11 and 30 days (control) and the root development periods were 22, 19 and 0 days respectively. Then plants were transferred to a tray in the same conditions as above.

2.4. Effect of brief exposures to low relative humidity

Rooted plants were transplanted to plastic pots with fertilized peat. Twenty five control plants were covered with plastic sheets and placed in a shaded greenhouse (60% shade). Thirty six plants were covered with inverted plastic bags which were removed at intervals. Half of them were placed in the shaded greenhouse and the rest were left in the culture chamber under 6 000 lx (light supplied by 'Sylvania' cool-white fluorescent tubes) and 16 h photoperiod. Bags were removed once a day at increasing periods according to the following ranges: 1st week 1.5-22 min; 2nd week 22 min- 1 h 30; 3rd week 1 h-4 h; 4th week 4 h-open. Dead plants were recorded every 5 days.

3. Results

3.1. Effect of agar concentration

The increase of agar concentration did not have a significant effect on the rooting percentage (table 1) but reduced significantly ($P \leq 0.05$) the number of roots per shoot and increased the survival rate at the highest agar concentration.

Table 1 - Effect of agar concentration on rooting and survival rate.

agar conc.	n° shoots	% rooting	n° roots/shoot	±s.e.	% survival
6 g/l	100	93 a	6.4 a	0.38	13.0 a
10 g/l	100	89 a	5.1 b	0.34	14.6 a
14 g/l	100	87 a	3.9 c	0.29	45.3 b

3.2. Effect of root developing medium

While a reduction in the root induction period decreased rooting percentage and number of roots per shoot (table 2), no effect on survival was detected ($P \geq 0.05$).

Table 2 - Effect of root developing medium on rooting and survival rate.

days in medium

(+) IBA	(-) IBA	n° shoots	% rooting	n° roots/shoots	±s.e.	% survival
30	0	84	98.9 a	8.5 a	0.50	16.7 a
11	19	80	100.0 a	6.8 b	0.32	31.2 a
8	22	80	92.5 b	6.6 b	0.48	24.3 a

3.3. Effect of brief exposures to low relative humidity

The survival percentage was low for control plants (figure 1, A) but was high for both exposed treatments (figure 1, B and C). Decline in survival rate in control plants began fifteen days after being transferred to pots.

Figure 1 - Survival percentage evolution in acclimatization processes. A) Standard acclimatization. B and C) Acclimatization with brief exposures to low relative humidity.

4. Discussion

While some authors (Cossio et al., 1981; Cossio, 1981; Snir, 1983) have not reported problems in acclimatization, others (Marín et al., 1983; Ponchia et al., 1980) obtain a poor survival rate despite the prevention of desiccation. Furthermore, plants began dying fifteen days after being transferred to pots. This fact questions desiccation as the main cause of death, as proposed by Sutter et al. (1979) and Brainerd et al. (1981), because desiccation would have affected plants earlier.

High agar concentrations in the rooting medium had an effect on the acclimatization process increasing the survival percentage, although not to the rate recorded by Zilis et al. (1979). It is possible that a reduced water availability preconditioned plants to the new environmental conditions.

The root development medium, without agar, was used by Snir (1983) to improve the rate of rooting after a root induction period. This medium was used to see its effect on the functioning of roots in acclimatization, but no significative influence was detected.

Plants exposed daily, at increasing periods, to low relative humidity and either low or high light intensity, had a high survival rate. This suggests that plants need stimulating to start the adaptive mechanisms required for the new environmental conditions and to the switch over to autotrophic life.

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