



***In vitro* propagation of peperina (*Minthostachys mollis* (Kunth.) Griseb.)**

Paula Bima, Laura Vargas and Marta Ojeda

Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Av. Valparaíso s/n, CC 509, Córdoba, Argentina. e-mail: pabima@agro.uncor.edu

INTRODUCTION

Among non-cultured aromatic native species, peperina is the most required and the only spontaneous species with international demand (Toursarkissian, 1980), thus presenting a high degradation level. In order to try resource conservation, a process of productive domestication was started, thus selecting plants that should be evaluated in clonal lots (Ojeda, 2004).

Through clonal multiplication of the best individuals evaluation may be performed in comparative tests of yield and economic rentability. Therefore, selected clones of commercial value would be obtained.

The *in vitro* propagation technique allows to obtain a great number of plants (clones) from selected initial material, and under the best sanitary conditions (Levy and Levy, 1991). It can be an economically profitable tool for garden nursery production of this and other aromatic species, contributing to preserve the resource in its natural habitat.

The outlined aim was to optimize experimental conditions for rapid *in vitro* multiplication techniques, and transference to soil of selected peperina clones.

MATERIAL AND METHODS

New shoots of a cultured clone were used under greenhouse conditions.

Step I: Shoot Disinfection: Two treatments were carried out:

(1) Washing with water for an hour, and then, Immersion in 70% ethanol for 10 sec, and 1.5% sodium hypochlorite + Tween 20 for 20 min.

(2) Immersion in 70% ethanol for 10 sec, and 1.5%

sodium hypochlorite + Tween 20 for 10 min.

Step II: Multiplication:

Shoots that developed a plantlet were multiplied by internode division (microcuttings). Therefore, Murashige and Skoog (MS) culture medium was used + 0.1 mg l⁻¹ BA + 0.5 mg l⁻¹ IBA.

Step III: *In vitro* rooting:

Uninodal and apical cuttings from the previous step were placed in MS basal medium with the addition of different combinations and concentrations of IBA (0.5; 1 and 1.5 mg l⁻¹) and sucrose (30; 40; 50 and 100 g l⁻¹).

Culture incubation conditions were 22°C of temperature and 16 h of photoperiod.

RESULTS AND DISCUSSION

Disinfection which consisted of shoot washing with tap water, and then immersion in 70% ethanol for 10 sec and sodium hypochlorite + Tween 20 for 20 min (treatment 1) allowed to obtain the highest shoot percentage without external contaminations (90% versus 53%).

Apical cuttings were the most efficient for rooting (Tables 1 and 2). The thickest and numerous roots were observed in treatments with 40 g/l of sugar. Roots developed within 7 days of culture in 80% of the plants, with 1 mg l⁻¹ and 1.5 mg l⁻¹ IBA. Peperina required an additional passage in a rooting medium without cytokinins and with a higher concentration of auxin, as, unlike oregano (Goleniowski *et al.*, 2003; Bima *et al.* 2004) it doesn't produce roots in abundance during the



multiplication step.

Table 1. Percentage of *in vitro* uninodal rooted cuttings in culture media combining different concentrations of IBA and Sucrose

| Sucrose | 30 g l ⁻¹ | | | 40 g l ⁻¹ | | | 50 g l ⁻¹ | | | 100 g l ⁻¹ | | |
|------------------------|----------------------|----|----|----------------------|----|-----|----------------------|----|----|-----------------------|----|----|
| | % rooted plants | | | % rooted plants | | | % rooted plants | | | % rooted plants | | |
| Culture days | 7 | 15 | 30 | 7 | 15 | 30 | 7 | 15 | 30 | 7 | 15 | 30 |
| IBA | | | | | | | | | | | | |
| 0.5 mg l ⁻¹ | 8 | 33 | 92 | 0 | 17 | 100 | 17 | 58 | 75 | 0 | 17 | 25 |
| 1.0 mg l ⁻¹ | 25 | 83 | 83 | 25 | 83 | 92 | 0 | 25 | 92 | 0 | 8 | 17 |
| 1.5 mg l ⁻¹ | 66 | 66 | 92 | 0 | 33 | 92 | 0 | 83 | 67 | 0 | 33 | 58 |

Table 2. Percentage of *in vitro* apical rooted cuttings in culture media combining different concentrations of IBA and Sucrose

| Sucrose | 30 g l ⁻¹ | | | 40 g l ⁻¹ | | | 50 g l ⁻¹ | | | 100 g l ⁻¹ | | |
|------------------------|----------------------|-----|-----|----------------------|----|-----|----------------------|----|-----|-----------------------|----|----|
| | % | | | % | | | % | | | % | | |
| Culture days | 7 | 15 | 30 | 7 | 15 | 30 | 7 | 15 | 30 | 7 | 15 | 30 |
| IBA | | | | | | | | | | | | |
| 0.5 mg l ⁻¹ | 75 | 75 | 75 | 33 | 67 | 100 | 67 | 92 | 92 | 25 | 50 | 50 |
| 1.0 mg l ⁻¹ | 50 | 100 | 100 | 75 | 83 | 92 | 58 | 92 | 100 | 25 | 50 | 50 |
| 1.5 mg l ⁻¹ | 50 | 50 | 75 | 83 | 92 | 100 | 50 | 92 | 100 | 25 | 58 | 58 |

Fig. 1: Peperina plants from *in vitro* culture, 30 days after transplanting into greenhouse.



CONCLUSIONS

The attainment of *in vitro* rooted acclimatized plants allowed with success transplant to greenhouse conditions. This will allow to multiply selected clones, and to use them for improvement programs or for homogeneous commercial productions, and of proved quality.



Note: This study was presented at the 'I Reunión de Biotecnología aplicada a plantas medicinales y aromáticas' (First Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2006.

REFERENCES

Bima P.; Torres L.; Flamarique M.C. y Cognigni W. 2004. *Micropropagación de orégano "Negrito" (Origanum spp.)*. In: XXVII Congreso Argentino de Horticultura. Merlo. San Luis. Resúmenes.

Levy L. W. and Levy P. E. (1991) *Propagación masiva del piretro y guanto mediante cultivo de tejidos*. In: *Cultivo de Tejidos en Agricultura, fundamentos y aplicaciones*. Chapter 29, Roca W. and Morginski L. A. eds., CIAT; 953 pp.

Goleniowski M.E., Flamarique M. C. and Bima P. (2003) Micropropagation of oregano (*Origanum vulgare* x *O. applii*) from meristem tips. *In vitro Cell. Dev. Biol. Plant* **39**: 125-128.

Ojeda M. (2004). Caracterización de poblaciones y avances en la domesticación de peperina *Minthostachys mollis* (Kunth.) Griseb. *Ph.D. Dissertation*, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba, Argentina; 130 pp.

Toursarkissian M. (1980) *Plantas medicinales de la Argentina, sus nombres botánicos, vulgares, usos y distribución geográfica*. Ed. Hemisferio Sur SA., Buenos Aires, Argentina; 177 pp.



Fig. 1: Peperina plants from *in vitro* culture, 30 days after transplanting into greenhouse.