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Program and Abstracts

Comparative study between in vitro and in vivo developing root systems in micropropagated chestnut

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Aim

The aim of this study was to compare two rooting environmental conditions for root development, in vitro and ex vitro, and their influence in anatomical differentiation.

Material and Methods

Plant material:	An adult *Castanea sativa* x *C. Crenata* hybrid clone, M3, resistant to ink disease, established *in vitro* two years ago. Establishment and
multiplication stages:	According to Vieitez *et al.*, 1986 and Gonçalves *et al.*, 1998.
Rooting:	Performed in two steps:
Induction:	*In vitro*, 5 days in 1/2MS1/4NO$_3$ medium + 6 gl^{-1} of activated charcoal and without auxin.
Expression:	*In vitro*: 28 days in 1/2MS1/4NO$_3$ medium + 3 mgl^{-1}
	In vivo: 28 days in a polystyrene boxes with a 70% hydrated peat:perlite (1:2, v:v) sterilised mixture, fertilised with 1/2MS1/4NO$_3$
Incubation conditions:	In a growth chamber with: Photoperiod: 16 hours;
Irradiance:	150 ± 10 mmol photons m^{-1} s^{-1}; Temperature: 25 ± 2 ºC; Humidity: ³ 95% RH.
Histology:	Material: root developed under in vitro and in vivo conditions after 28 days of rooting.
Fixation:	In 35% formalin : glacial acetic acid : 50% ethanol (5:5:90, v:v:v).
Dehydration:	In ethanol series between 60 and 95%.
Embedding:	In LKB Historesinâ, Leica Comercialâ, Kit 70-2218-500.
Cut:	Serial 7 mm thick transverse sections.
Staining:	With periodic acid Schiff (PAS) reaction and toluidine blue counterstained.

Fig. 1. Root induction in 3 mgl^{-1} IBA during 5 days.

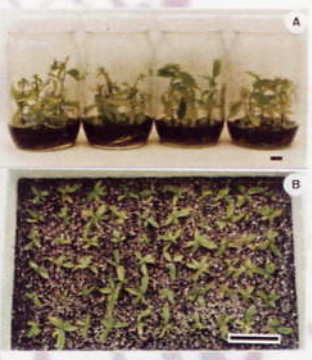

Fig. 2. Root developing and expression systems. (A) *In vitro*, with a 1/2MS1/4NO$_3$ medium + 6 gl^{-1} of activated charcoal and without auxin. (B) *In vivo*, in a polystyrene boxes with a 70% hydrated peat:perlite (1:2, v:v) sterilised mixture, fertilised with 1/2MS1/4NO$_3$.

Results and Discussion

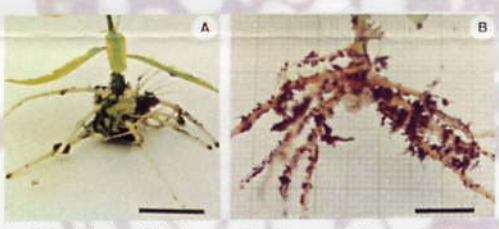

Fig. 3. Amplification of the shoot root junction area. (A) *In vitro developed root system. (B) In vivo developed root system. Bar represents 1 cm.*

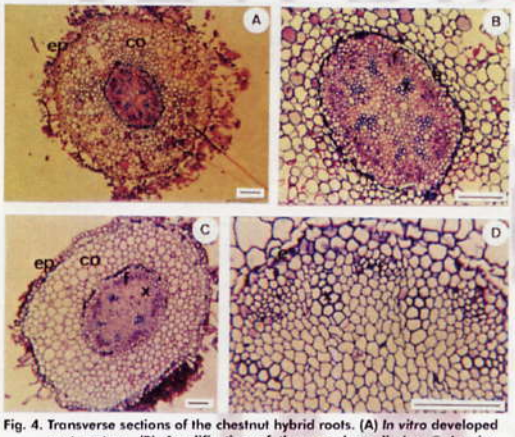

Fig. 4. Transverse sections of the chestnut hybrid roots. (A) *In vitro* developed root system. (B) Amplification of the vascular cylinder. © *In vivo* developed root system. (D) Amplification of the vascular cylinder. Bar represents 100 mm in A, B and C, and 50 mm in D.

The morphological and anatomical aspects of the *in vitro* and *in vivo* developed roots are quit different. In vitro developed roots shown a significant callus in stem / root junction areas, lacked branch roots and have a reduced number of root hairs. On the other hand, the in vivo developed roots shown a quite normal pattern, with a total absence of callus formation and a branched and numerous root hairs. In transverse sections, the cortex and vascular areas of the in vitro roots shown significant intercellular spaces and a deficient organization with an abnormally large cross-sectional ratio between them. The in vivo developed roots have their cortical cells more regular and well organized, with insignificant intercellular spaces. In the vascular cylinder, the xylem and phloem tissues are more differentiated. Also the cross-sectioned ratio between cortex and vascular system are reduced. Other significant and important difference reported between these two root systems was that some in vivo developed roots shown, frequently, differentiation of secondary anatomical structures. In fact was clearly visible the vascular cambium differentiation and correspondent secondary xylem and phloem cells.

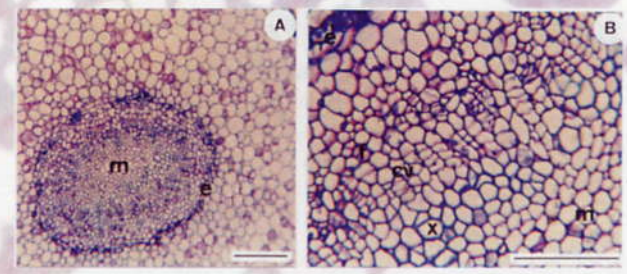

Fig. 5. Transverse section of *in vivo* developed root. (A) Vascular cylinder. (B) Amplification of the vascular cambium area. Bar represents 100mmin A and 50mm in B.

Final Remarks

This work shows that the morphological and anatomical study was significantly affected by the environmental developing root systems conditions. Of the two environments conditions, in vitro and in vivo, the last one allows the differentiation of a more normal anatomical root pattern, which have a significant influence of the physiological behaviour of these micropropagated chestnuts during acclimatization (Gonçalves *et al.*, 1998).

References

Gonçalves, J.C.; Diogo, G.; Amâncio, S. (1998). *In* vitro propagation of chestnut (*Castanea sativa* x *C. Crenata*) Effects of rooting treatments on plant survival, peroxidaes activity and anatomical changes during adventitious root formation. Sci. Horticulturae 72:265-275.
Vieitez, A.M.; Vieitez. M.L.; Vieitez, E. (1986). Chestnut (Castanea spp). In: Bajaj, Y.P.S. (ed.), Biotechnology in Agriculture and Forestry, Vol. 1: Trees. Springer-Verlag, Berlin, pp. 393-414.